Volume 33 JULY-AUGUST 1953 No. 4

CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(formerly *Scientific Agriculture*)

CONTENTS

EDITED BY THE
AGRICULTURAL INSTITUTE OF CANADA
OTTAWA 4, CANADA

Published bi-monthly by the authority of the Chairman of the National Committee on Agricultural Services

CORRESPONDENCE AND SUBSCRIPTIONS

Address all correspondence to the Agricultural Institute of Canada, Grindley Hall, 338 Somerset Street West, Ottawa 4, Canada. *Canadian Journal of Agricultural Science,* (formerly *Scientific Agriculture*) will be mailed to any address in Canada, the United States, Mexico, Central and South America and the British Commonwealth for $3.00 a year; other countries $4.00 a year. Single copies, 50 cents.

Authorized as second class mail, Post Office Department, Ottawa.

CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(*formerly* Scientific Agriculture)

VOLUME 33 JULY–AUGUST, 1953 No. 4

THE EFFECT OF 2,4-D ON THE DEVELOPMENTAL PROCESSES IN BARLEY AND OATS[1]

GEORGE FRIESEN[2] AND P. J. OLSON[3]

The University of Manitoba, Winnipeg, Man.

[Received for publication October 17, 1952]

ABSTRACT

Barley and oats were sprayed with 2,4-D in the greenhouse at 1- or 2-day intervals from emergence until after heading. Photomicrographs were made at each spraying date to determine the stage of development of the growing point. In addition, oats were treated in the field at 3-day intervals between pre-emergence and post-heading.

In barley there were two critical periods. The first was a seedling period, coinciding with the incidence of leaf initials and the differentiation of the tiny spike in the growing point. Leaf deformities characterized the early part of this period, and spike deformities the latter part. The second period began in the advanced boot stage, and continued until just before spike emergence. Sterility induced at this time occurred during the differentiation of anthers and stigma and early growth of the floral parts.

The incidence of abnormalities in oats corresponded with that in barley, except that in oats leaf deformities appeared over a much longer period of time. Anther differentiation began much earlier and pistil differentiation relatively later in relation to inflorescence differentiation in oats than in barley.

Previous investigations have shown that barley is highly susceptible to damage from 2,4-D during two periods in its development. The first extends from seedling emergence until the primary shoot has three or four leaves and has reached a height of 5 or 6 inches (general field level). The second begins when the plants are well advanced in the boot stage, 12 to 14 days before heading, and continues until heading. In oats the situation is not quite as clear, but in general the first susceptible period continues somewhat later in relation to external plant development, continuing to or almost to the early boot stage. The early seedling stages show the same type of leaf abnormalities, mainly tubular or onion-like leaves, as in barley.

The object of the present investigation was to relate the yield and morphological responses of barley and oats to the stage of morphological development of the plants at the time of 2,4-D treatment. The growth stages were studied and described, both on the basis of external morphological development of the plant, and by dissection and micro-examination of the growing point.

REVIEW OF LITERATURE

Andersen and Hermansen (1) applied sodium salt of 2,4-D (also a sodium salt of 4K-2M), to barley and oats twice a week and once a week,

[1] Contribution from the Division of Plant Science, The University of Manitoba, Winnipeg, Man.

[2, 3] Graduate Assistant and Professor, respectively, Division of Plant Science, The University of Manitoba. Adapted from a thesis submitted by George Friesen in partial fulfilment of the requirements for the degree of Master of Science.

respectively. They found that leaf abnormalities (tubular leaves mainly), resulted from treatment applied immediately after sprouting. Spike and panicle abnormalities appeared in greatest numbers following treatment applied 8 days later in the case of barley, and 17 days later in oats.

Olson *et al.* (9) found that, in wheat and barley, there were two widely separated periods during which damage was done by 2,4-D. The first was an early seedling and the second a late pre-heading period.

Derscheid (5) determined the stage of development of the growing point of barley treated with 2,4-D throughout most of its growing period. He found that during the period of greatest susceptibility, which occurred before the 5-leaf stage, differentiation of tiller buds was inhibited. During a second susceptible period occurring between pre-heading and late heading stages, the number of seeds per spike was reduced.

Friesen and Harris (6), Friesen *et al.* (7), Davidson (4) and Leggett (8) conducted a coordinated set of experiments in which oats were treated with an ester, and, in some cases, also an amine. The results reported agreed in showing that the greatest reduction in yield took place following treatments applied over a period starting when the plants were 6-7 inches tall and continuing to or almost to the boot stage. Leaf and panicle abnormalities were conspicuous results of the earlier seedling stage treatments. These were accompanied by some reduction in yield in some cases.

EXPERIMENTAL PROCEDURE

The plan of this investigation was to apply 2,4-D to Montcalm barley and Ajax oats in the greenhouse at different stages of growth, beginning between seeding and emergence and continuing at 1- or 2-day intervals until after heading. The barley experiment contained 32 treatment and 8 check or control pots; the oat experiment, 30 treatment and 6 check pots, randomized in each case in each of six replicates. An isopropyl ester of 2,4-D was used on barley and a butyl ester on oats. The rate applied was 12 ounces acid equivalent per acre in 120 gallons of water. Spraying was done with a small hand sprayer.

PLATE 1

A. Growing point of barley at treatment date 1, showing production of leaf initials. Pre-emergence. (X 24).

B. Growing point of barley at treatment date 2, showing production of leaf initials. 1-leaf, stage, plants 3 inches tall. (X 24).

C. Growing point of barley at treatment date 8, showing its elongation shortly before spike differentiation. (8 days after emergence). Plants 7 inches tall; 2-leaf stage. (X 40).

D. Growing point of barley at treatment date 9, showing spike differentiation. Plants 8 inches tall; 3-leaf stage. (X 12).

E. Developing spike of barley at treatment date 12, showing spikelet differentiation. (15 days after emergence). Plants 13 inches tall; 4-leaf stage. (X 40).

F. Developing spike of barley at treatment date 15, with lemma and palea clearly visible. (18 days before heading). Plants 17 inches tall; 5-leaf stage.

G. Developing spike of barley at treatment date 17. Reproductive organs forming in young florets. (14 days before heading). Plants 20 inches tall; 6-7-leaf stage. (7th leaf = fly leaf.)

H. Developing spikes of barley at treatment dates 18 to 20. (12 to 9 days before heading).

A
B
C
D
E
F
G
H

A
B
C
D
E
F
G
H

The stage of development of the main culms was recorded at each spraying date; both as to the external morphological development (height in inches, number of leaves, relation to emergence or heading, etc.), and by examination of the growing point. Dissections, photomicrographs, and interpretation of stage of development, were made as outlined by Bonnett (2) (3).

At harvest-time individual plants were studied carefully with regard to the incidence of various types of abnormalities following treatment at the different stages. Observations were made separately on the main culms and tillers and photographs were made of all the abnormalities studied.

Yields were also determined separately for the main culms and tillers. In barley each plant, for the most part, had two tillers (in addition to the main culm) that produced mature spikes, while in oats three tillers produced mature panicles.

Temperature was controlled and kept near 75 degrees in the daytime and 60 degrees at night. Although no special effort was made to control humidity, it remained near constant at 65 per cent.

RESULTS AND DISCUSSION

Plate 1 shows several stages in the development of the growing point and spike of barley. The respective morphological stages of development are also indicated. The development of the growing point could be divided into three phases. During the first phase the growing point remained short and only leaf initials were being produced. This phase commenced with germination and continued until 4 days after emergence when the plants were $5\frac{1}{2}$ inches tall (stretched) and had two leaves. During the second phase the undifferentiated portion of the growing point elongated in preparation for spike development. The growing point remained smooth in outline and tiller buds were formed in the axils of the leaves. This stage continued until 8 days after emergence when the plants were 7 inches tall (stretched) and had three leaves (see C, *Plate 1*). The beginning of the

---

PLATE 2

A. Growing point of oats at treatment date 1, showing production of leaf initials. Pre-emergence. (X 32).

B. Growing point of oats at treatment date 3, showing production of leaf initials. Plants $1\frac{1}{2}$ inches tall; 1-leaf stage. (X 32).

C. Growing point of oats at treatment date 6, showing production of leaf initials and tiller primordia. Plants 5 inches tall; 2-leaf stage. (X 32).

D. Growing point of oats at treatment date 12, showing small depressions on elongated growing point. (19 days after emergence). Plants 11 inches tall; 3-leaf stage. (X 32).

E. Growing point of oats at treatment date 16, showing branching of panicle beginning. (29 days after emergence). Plants 17 inches tall; 4-leaf stage. (X 32).

F. Developing panicle of oats at treatment date 17, showing developing lemmas. (21 days before heading). Plants 18 inches tall; 5-leaf stage. (X 32).

G. Developing panicle of oats at treatment date 19, showing spikelet differentiation. 16 days before heading. Plants in 6-leaf stage. (X 32).

H. Developing panicle of oats at treatment date 21. 10 days before heading. 7-8-leaf stage. (8th leaf = flag leaf).

third phase was marked by the appearance of double ridges on the growing point, followed by the differentiation and development of the spike and its parts. The order of differentiation of the various parts of the spike as far as could be seen in this study were: spikelet initials, glumes, lemma and palea, awns, and reproductive parts.

Plate 2 shows several stages in the development of the growing point and panicle of oats. Again the respective stages of morphological development are indicated. The growing point of oats, like that of barley, passes through three phases in its development. During the first phase the growing point remained short, the leaf initials differentiated, and tiller buds developed in the axils of the leaves at the base of the stem. This first phase was observed up to 15 days after emergence of the plants. During the second phase the growing point elongated (see D, *Plate 2*), but the elongation was much less marked than in barley. During the third phase the internodes of the stem elongated and the branches, spikelets, and flower parts differentiated and developed. Panicle formation was first indicated by the appearance of single, lateral, alternate projections arising beneath the apex of the growing point (see E, *Plate 2*). Spikelets differentiated from the tips of the branch primordia and the empty glumes were the first of the spikelet parts to differentiate. The first sign of panicle differentiation was noted in plants 23 days after emergence. Flower parts differentiated in the following order; lemma, anthers, palea, lodicules and pistil.

Effect of the Treatments on Abnormalities in Barley

The pots that were treated just before emergence produced culms and tillers that were normal in all respects. Those that were treated 1 to 4 days after emergence showed leaf deformities on all or nearly all of the main culms. The most conspicuous of these were tubular or onion-like leaves; others showed fusion of two leaves, leaves with double midribs and supernumerary leaves. The spikes emerged with difficulty and many were completely sterile. The tillers produced in these pots were quite normal in all respects. The growing point, during this period, was in its first phase and was showing only leaf primordia. A, *Plate 3*, shows some of the leaf deformities observed.

Beginning with the plants treated 4 days after emergence, spike abnormalities appeared at heading time on some of the main culms. Those treated 5, 6, 8 and 9 days after emergence showed spike abnormalities on all main culms. These abnormalities consisted of branched spikes, supernumerary spikelets, "tweaking" (elongated internodes and opposite spikelets), twisted and twinned kernels, fused awns and lemmas, etc. The last of these deformities appeared in the pots treated 11 days after emergence. The first visible evidence of spike differentiation appeared in the plants 8 days after emergence. From here on, spike differentiation became quite clear. In the meantime leaf deformities began to appear on the tillers. Their appearance on the tillers, therefore, coincided with their disappearance on the main culms. The tillers began to show spike deformities in the pots treated 9 days after emergence. B to F, *Plate 3*, show some of the spike deformities observed.

PLATE 3

Deformities of barley caused by 2,4-D treatment.

A. Leaf deformities. (Left to right), normal leaf, onion-like or tubular leaves, incomplete heading resulting from the tubular leaf.

B. Spike deformity showing "tweaking"; elongated rachis internodes and spikelets opposite instead of alternate on the rachis.

C. Spike deformity showing supernumerary spikelets at each node resulting in a clubbed appearance.

D. Spike deformities. (Left to right), supernumerary spikelets, branched spike, double branched spike.

E. Spike deformity. Twinned or double kernels. Many of these have been caused by fused lemmas.

F. Spike deformity. "Tweaking"; elongated internodes and spikelets opposite instead of alternate on the rachis.

G. Complete sterility. At left, normal spike; at right, spike that is morphologically normal but completely sterile.

H. Complete sterility. Spike at right is partially removed to show its position relative to the flag-leaf.

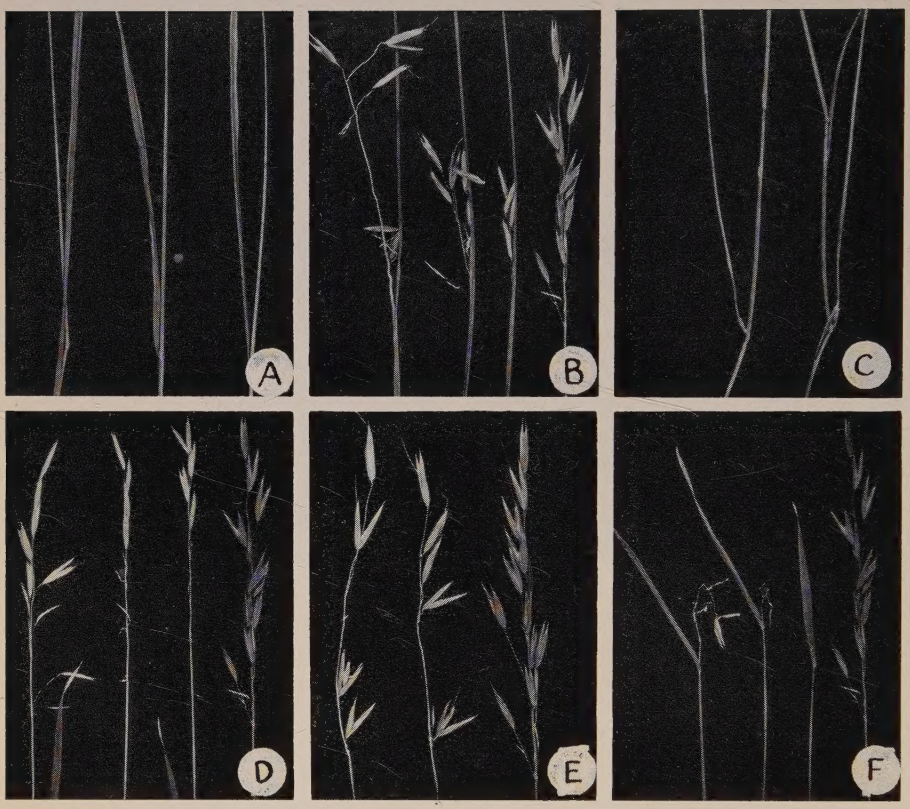


PLATE 4

Deformities of oats caused by 2,4-D treatment.

A. Tubular or onion-like leaves.
B. Incomplete heading due to onion-like leaves. (Normal panicle at right).
C. Aerial tillering.
D. Basal sterility. (Normal panicle at right).
E. Central sterility. (Normal panicle at right).
F. Complete sterility. (Normal panicle at right).

The plants receiving the 11th to 16th treatments produced main culms that were normal in all respects. During this period the plants advanced from a height of 12 inches (stretched) to 18 inches and had three and six leaves, respectively. By this time the spike was one inch above ground level and the lemma, palea and awns were clearly visible in the dissected plants.

Beginning with the 17th treatment date some sterility was induced in the main culms. This increased, becoming complete or practically so, in the pots receiving the 19th treatment and continuing through the 23rd treatment, which was applied 4 days before heading. This corresponded to early stages of development in floral parts, anthers, ovary, style and stigma, and continued until fertilization had taken place. The tillers at this time remained normal. From the 24th treatment, applied 2 days before full heading, through the final or 32nd treatment date, no sterility was induced in the main culms. During this period the treatments caused

TABLE 1.—THE PERCENTAGE OF BARLEY PLANTS EXHIBITING VARIOUS TYPES OF ABNORMALITIES DUE TO TREATMENT WITH 2,4-D (ISOPROPYL ESTER). CHEMICAL APPLIED AT THE RATE OF 12 OUNCES PURE ACID PER ACRE, AT 32 DIFFERENT STAGES OF GROWTH. (FOR STAGE OF GROWTH SEE TABLE 3)

Treatment date	Leaf deformities		Spike deformities		Complete sterility	
	Main culms	Tillers	Main culms	Tillers	Main culms	Tillers
1	—	—	—	—	—	—
2	78.0	—	—	—	—	—
3	56.0	—	11.0	—	—	—
4	84.0	—	16.0	—	—	—
5	61.0	5.0	39.0	—	—	—
6	11.0	20.0	83.0	10.0	—	—
7	6.0	33.0	94.0	19.0	—	—
8	—	26.0	100.0	24.0	—	—
9	—	40.0	100.0	44.0	—	—
10	—	16.0	78.0	55.0	—	—
11	—	5.0	11.0	90.0	—	—
12	—	—	6.0	100.0	—	—
13	—	—	—	72.0	—	—
14	—	—	—	46.0	—	—
15	—	—	—	27.0	6.0	—
16	—	—	—	33.0	22.0	—
17	—	—	—	13.0	44.0	—
18	—	—	—	—	72.0	—
19	—	—	—	—	83.0	—
20	—	—	—	—	83.0	—
21	—	—	—	—	72.0	—
22	—	—	—	—	89.0	7.0
23	—	—	—	—	56.0	11.0
24	—	—	—	—	—	16.0
25	—	—	—	—	—	14.0
26	—	—	—	—	—	15.0
27	—	—	—	—	—	18.0
28	—	—	—	—	—	16.0
29	—	—	—	—	—	24.0
30	—	—	—	—	—	26.0
31	—	—	—	—	—	22.0
32	—	—	—	—	—	18.0

a high degree of sterility in the tillers. G and H, *Plate 3*, show two types of sterility caused by 2,4-D at these stages.

Table 1 shows the per cent of main culms and tillers exhibiting the various types of deformities studied.

The results of Andersen and Hermansen (1), as regards type of abnormalities in relation to stage of growth, agree closely with those reported here. They point out that "tubular" leaves occur when treatment takes place immediately after sprouting. Abnormalities of the spike are produced by later spraying. On May 4, their second date of spraying, the plants were 13 cm. in height and the length of the spike was 0.48 mm. Treatment on this date caused the greatest number of spike abnormalities, the number of abnormal spikelets reaching 42.7 per cent of the total under the 4 Kg. treatment. Shortly before heading there was another susceptible period which Andersen and Hermansen considered to be the time when sex cells began to develop.

Derscheid (5) found in his investigation that such abnormalities as constricted sheath (tubular leaf) and incomplete heading were prevalent on the plants treated before "floral initiation" occurred, whereas spike abnormalities were most prevalent on plants treated during the period of floral initiation. The period so designated corresponds with that described in this paper as the period of "spikelet and floret differentiation". There is excellent agreement, therefore, between Derscheid's results and those reported here. Derscheid also found that "blasted" florets were most common on plants treated near heading time, corresponding to the high degree of "sterility" reported in this paper for the treatments applied during a period of 12 to 4 days before heading. Derscheid suggested that inhibition of embryo sac and gamete development might have been responsible for this result. These results support and complement each other in indicating that floral primordia are particularly susceptible to 2,4-D and that this chemical is also highly detrimental to fertilization during anthesis. They indicate further that the meristematic tissues of any plant organs are highly susceptible to damage from 2,4-D.

The Effect of the Treatment on Abnormalities in Oats

The plants that were treated before emergence produced main culms and tillers that were normal in all respects. Those that were treated 1 to 15 days after emergence showed leaf or vegetative deformities on all or nearly all of the plants. The most conspicuous of these were tubular or onion-like leaves. Others showed aerial tillering, twisted stems and supernumerary leaves. The panicles emerged with difficulty and many were completely sterile. The growing point during this period was showing only leaf initials. Tiller primordia were evident on plants 5 days after emergence. The tillers produced these leaf deformities on plants sprayed 5 to 26 days after emergence of the main culms. A and B, *Plate 4*, show some of the leaf or vegetative deformities studied.

Beginning with the plants treated 14 days after emergence panicle abnormalities appeared at heading time on some of the main culms. They appeared in greatly increased numbers following the next two treatments. These consisted of basal and central sterility, non-split glumes and branching at the uppermost node. The first visible evidence of panicle differ-

entiation appeared on plants treated 23 days after emergence, and from here on panicle differentiation became quite clear. The tillers began to show these panicle deformities in the pots treated 17 days after emergence and their appearance continued until the 19th treatment date (36 days after emergence). C-E, *Plate 4*, show some of the panicle abnormalities observed.

The plants receiving the 14th to 16th treatments produced main culms that were normal in all respects. During this period the plants advanced from a height of $13\frac{1}{2}$ inches (stretched) to 17 inches and had four to five leaves. At this time the panicle was 1 inch above the ground level and the empty glumes were beginning to show in dissected plants. Except for a few panicle deformities, the tillers were normal in the pots receiving the 18th and 19th treatments.

Beginning with the 17th treatment some plants showed complete sterility in the main culms. This increased and, in pots receiving the 20th and 21st treatments, over 50 per cent of the panicles on the main culms were completely sterile. This period corresponded to early stages in the development of the reproductive parts and continued until fertiliza-

TABLE 2.—SHOWING THE PERCENTAGE OF OAT PLANTS EXHIBITING VARIOUS TYPES OF ABNORMALITIES DUE TO TREATMENT WITH 2,4-D (BUTYL ESTER). CHEMICAL APPLIED AT THE RATE OF 12 OUNCES PURE ACID PER ACRE, AT 30 DIFFERENT STAGES OF GROWTH. (FOR STAGE OF GROWTH SEE TABLE 4)

Treatment date	Leaf deformities		Spike deformities		Complete sterility	
	Main culms	Tillers	Main culms	Tillers	Main culms	Tillers
1	—	—	—	—	—	—
2	66.7	—	—	—	—	—
3	100.0	—	—	—	—	—
4	94.4	4.3	—	—	—	—
5	100.0	17.6	—	—	—	—
6	100.0	46.9	—	—	—	—
7	100.0	64.1	—	—	—	—
8	94.4	69.0	—	—	—	—
9	72.2	75.7	22.2	—	—	—
10	38.9	55.3	72.2	—	—	—
11	22.2	53.6	77.8	10.7	—	—
12	—	44.7	38.9	26.3	—	—
13	—	44.4	11.1	28.9	—	—
14	—	16.7	—	57.1	—	—
15	—	26.7	—	50.0	—	—
16	—	10.0	—	52.5	—	—
17	—	5.3	—	55.3	5.6	—
18	—	—	—	20.9	27.8	—
19	—	—	—	10.2	38.9	4.1
20	—	—	—	7.5	61.1	22.5
21	—	—	—	4.8	55.6	23.8
22	—	—	—	—	33.3	22.6
23	—	—	—	—	5.6	42.0
24	—	—	—	—	—	18.9
25	—	—	—	—	—	4.9
26	—	—	—	—	—	4.7
27	—	—	—	—	—	1.6
28	—	—	—	—	—	—
29	—	—	—	—	—	—
30	—	—	—	—	—	—

tion had taken place. From the 24th treatment, applied 3 days before heading, through the final or 30th treatment, no sterility was induced in the main culms. Following treatments 20 to 26, inclusive (13 days before heading to 4 days after heading in the main culm) a high degree of sterility was recorded in the tillers.

Table 2 shows the percentage of oat plants exhibiting the various types of abnormalities in the main culms and tillers. It can be seen that the occurrence of abnormalities in oats paralleled closely those in barley with the exception that the leaf deformities appeared over a longer period of time. In oats the leaf deformities resulted from treatment within 15 days after emergence of the crop while in barley no onion-like leaves were observed in the main culms when treated 6 days after emergence. This was followed in both crops by spike or panicle deformities. These results are similar to those reported by Andersen and Hermansen (1) who found that spike deformities were most numerous in barley when treated 8 days after sprouting while in oats panicle deformities were most prevalent when treated 17 days after sprouting.

The Effect of the Treatments on Yields in Barley

Sharp yield reductions in the main culms took place as a result of the treatments applied during about a 4-day period after emergence. This was during the single leaf stage and corresponded to the period when leaf abnormalities were induced. Some reduction also took place following the treatments applied during the 5th to the 9th days after emergence, extending over the two- into the three-leaf stage, a period during which spike abnormalities were induced. The greatest yield reduction in the tillers took place in the pots treated during this latter period. Evidently the tillers were in the same stage of development during this period as the main culms were during the preceding one.

The greatest yield reduction in the main culms took place in the pots treated 14 to 4 days before heading. This was the period during which virtually complete sterility was induced in the spikes produced on the main culm. Reduction in yield of tillers occurred over a period of several days immediately following, corresponding to a high degree of sterility in the tillers.

When the combined yields of main culms and tillers were determined it was found that the greatest reduction took place in the pots treated from the 5th to 9th days after emergence, corresponding exactly with the period when spike deformities were induced in the main culms and leaf deformities in the tillers. The reduction at the late pre-heading stages was somewhat less in degree, and corresponded with the period of greatest reduction in the main culm yields. Table 3 shows the yields for the main culms, tillers and total yield.

Applying these results to field conditions the degree of yield reduction, and the range of the periods of yield reduction would depend upon the extent to which environment influenced tiller production. If conditions were unfavourable for tiller development, so that most plants consisted of single main culms, sharp yield reductions would be expected as a result of treatment soon after emergence and some reduction by later treatments during the two-leaf and three-leaf stages. If, on the other hand, conditions

TABLE 3.—EFFECT OF TREATMENT WITH 2,4-D (ISOPROPYL ESTER) ON THE YIELD OF BARLEY. TREATMENTS MADE AT 32 STAGES OF GROWTH AT THE RATE OF 12 OUNCES ACID EQUIVALENT PER ACRE

Treatment date	Stage of growth	Yields		
		Main culms	Tillers	Total
1	Pre-emergence	25.1	27.0	52.1
2	1½ inch, 1 leaf, 1 day a.e.[2]	3.9[1]	29.2	33.1
3	3 inch, 1 leaf, 2 days a.e.	9.4[1]	30.2	39.6
4	4½ inch, 1 leaf, 3 days a.e.	5.8[1]	33.9[1]	39.7
5	5½ inch, 2 leaves, 4 days a.e.	10.3[1]	32.8[1]	43.2
6	6 inch, 2 leaves, 5 days a.e.	14.5[1]	13.0	27.5[1]
7	6½ inch, 2 leaves, 6 days a.e.	14.6[1]	16.7	31.3[1]
8	7 inch, 2 leaves, 8 days a.e.	13.8[1]	17.8	31.6[1]
9	8 inch, 3 leaves, 9 days a.e.	11.3[1]	12.4	23.7[1]
10	11 inch, 3 leaves, 11 days a.e.	14.2[1]	19.3	33.5
11	12 inch, 3-4 leaves, 13 days a.e.	20.4	15.5	35.9
12	13 inch, 4 leaves, 15 days a.e.	14.4[1]	26.1	40.5
13	15 inch, 4 leaves, 17 days a.e.	12.5[1]	23.9	36.3
14	16 inch, 5 leaves, 19 days a.e.	13.3[1]	24.2	37.5
15	17 inch, 5 leaves	12.4[1]	21.9	34.3
16	18 inch, 6 leaves	11.1[1]	25.7	36.8
17	6-7 leaves, 14 days b.h.[3]	6.8[1]	27.7	34.5
18	7 leaves, 12 days b.h.	2.5[1]	25.6	28.1[1]
19	7 leaves, 11 days b.h.	0.7[1]	31.8[1]	32.5[1]
20	7 leaves, 9 days b.h.	1.0[1]	33.2[1]	34.2
21	7 leaves, 7 days b.h.	1.9[1]	28.7	30.6[1]
22	7 leaves, 6 days b.h.	0.6[1]	31.7[1]	32.3[1]
23	7 leaves, 4 days b.h.	5.4[1]	33.9[1]	45.3
24	7 leaves, 2 days b.h.	20.9	18.5	39.4
25	Heading stage	23.5	24.0	47.5
26	2 days past heading	26.8	19.4	46.2
27	4 days past heading	20.9	20.2	41.1
28	6 days past heading	25.1	21.2	46.3
29	8 days past heading	29.9	26.1	56.0
30	11 days past heading	26.8	24.6	51.4
31	13 days past heading	19.5	20.7	40.1
32	16 days past heading	24.3	18.1	42.3
Average yield of check pots		23.8	20.4	44.2
L.S.D. at 5 per cent level		6.6	11.2	11.7

[1] Difference significant at 5 per cent level.
[2] Days after emergence.
[3] Days before heading.

favoured tiller development, there might be little or no reduction resulting from the earlier treatments in the single leaf stage because the tiller yields would offset the damage caused to the main culm. There would be a sharp yield reduction from treatments applied in the two- and early three-leaf stages because the damage here would be a combination of the lesser main culm damage and the severe tiller damage.

The reduction in yield resulting from treatment in the pre-heading stages would be very sharp if there were little or no tiller development and would probably be greater than the seedling stage reduction because of the very high degree of sterility induced by treatments at these stages. Favourable conditions for tillering would reduce the apparent damage during this period and would extend its range. Actually, damage in the field has

usually taken place during late pre-heading stages. This may be due to difficulty in distinguishing between the tiller spikes and the main culm spikes at this time.

Effect of the Treatments on Yield in Oats

In the main culms significant yield reductions took place as a result of all treatments applied between emergence and heading. However, two periods stand out as being particularly low in yield. The first extends from 1 to 14 days after emergence (seedling leaf to early three-leaf stage) and corresponds to the period when leaf abnormalities were being caused. Difficulty in panicle emergence, with the resulting high degree of sterility, appears to account for the exceptionally low yields at this time. The second period includes treatments 16 to 22 (4-leaf stage to early flag-leaf stage) at which time many panicles showed complete sterility. In field

TABLE 4.—EFFECT OF TREATMENT WITH 2,4-D (BUTYL ESTER) ON THE YIELD OF OATS. TREATMENTS MADE AT 30 STAGES OF GROWTH AT THE RATE OF 12 OUNCES ACID EQUIVALENT PER ACRE

Treatment date	Stage of growth	Yields		
		Main culms	Tillers	Total
1	Pre-emergence	12.1	38.6	50.7
2	½ inch, 1 leaf, 1 day a.e.[2]	2.8[1]	23.5[1]	26.3[1]
3	1½ inch, 1 leaf, 2 days a.e.	0.5	17.7[1]	18.2[1]
4	2½ inch, 1 leaf, 3 days a.e.	0.2[1]	20.1[1]	20.4[1]
5	3½ inch, 1-2 leaves, 4 days a.e.	0.7[1]	11.7[1]	12.4[1]
6	5 inch, 2 leaves, 5 days a.e.	0.2[1]	12.0[1]	12.2[1]
7	5½ inch, 2 leaves, 6 days a.e.	3.3[1]	14.8[1]	18.0[1]
8	6 inch, 2 leaves, 8 days a.e.	2.7[1]	10.7[1]	13.4[1]
9	8 inch, 3 leaves, 9 days a.e.	3.8[1]	16.2[1]	20.0[1]
10	9 inch, 3 leaves, 11 days a.e.	6.0[1]	12.7[1]	18.7[1]
11	10 inch, 3 leaves, 13 days a.e.	5.4[1]	13.4[1]	18.7[1]
12	11 inch, 3 leaves, 15 days a.e.	6.6[1]	18.4[1]	25.0[1]
13	12 inch, 4 leaves, 17 days a.e.	6.3[1]	18.7[1]	25.0[1]
14	13½ inch, 4 leaves, 19 days a.e.	6.5[1]	22.9[1]	29.4[1]
15	16 inch, 4 leaves	5.2[1]	16.4[1]	21.5[1]
16	17 inch, 4 leaves	2.6[1]	18.0[1]	20.6[1]
17	18 inch, 5 leaves	3.3[1]	17.5[1]	20.8[1]
18	21 inch, 5 leaves	1.7[1]	19.7[1]	21.4[1]
19	6 leaves, 16 days b.h.[3]	4.3[1]	20.3[1]	24.6[1]
20	7 leaves, 13 days b.h.	3.6[1]	13.2[1]	16.8[1]
21	7-8 leaves, 10 days b.h.	1.6[1]	15.3[1]	16.9[1]
22	8 leaves, 7 days b.h.	3.8[1]	20.2[1]	24.0[1]
23	8 leaves, 5 days b.h.	6.1[1]	20.6[1]	26.7[1]
24	8 leaves, 3 days b.h.	7.6[1]	18.2	25.8[1]
25	Heading stage	11.6	15.7[1]	27.1[1]
26	4 days past heading	10.8	22.0[1]	32.9[1]
27	8 days past heading	13.9	32.3	46.2
28	11 days past heading	11.3	28.7	40.0
29	13 days past heading	9.2	38.5	47.7
30	16 days past heading	9.6	38.1	47.7
Average yield of all check pots		11.8	33.3	45.1
L.S.D. 5 per cent level		3.35	8.98	9.32

[1] Difference significant at 5 per cent level.
[2] Days after emergence.
[3] Days before heading.

experiments the most serious yield reductions have usually occurred at this time. In this respect oats differ from barley, the latter being resistant at the corresponding stage of external morphological development.

Significant reductions in tiller yields occurred as a result of treatment from emergence to 4 days past heading. Again the most serious reductions occurred as a result of treatment during the early stages, that is, when leaf deformities were being caused. Significant yield reductions were recorded in the tillers for those treatments that were applied immediately following emergence of the plants. This seems to indicate that, in oats, tiller primordia are differentiated very early in the developmental process. This was not the case in barley, where no damage in the tillers was recorded for treatments applied within 5 days after emergence of the plants.

The combined yields of main culms and tillers followed the pattern of the individual yields, with the most serious reductions corresponding to the periods when both main culms and tillers showed exceptionally low yields.

Table 4 shows the yields for the main culms, tillers and total yield for each treatment.

TABLE 5.—EFFECT OF TREATMENT WITH 2,4-D (ISOPROPYL ESTER) ON OATS. TREATMENTS MADE IN 1950 AT 3-DAY INTERVALS (BEGINNING AT PRE-EMERGENCE AND CONTINUING PAST THE HEADING STAGE). CHEMICAL APPLIED AT THE RATE OF 8 OZ. ACID EQUIVALENT PER ACRE

Date of application	Stage of growth	Abnormalities	Yield bu./ac.
June 6	Pre-emergence [1]	Normal	41.3
10	Pre-emergence [1]	Trace of onion-like leaves	46.9
13	2 inches	Many onion-like leaves. Erect growth	37.0
16	2½ inches	Many onion-like leaves. Very erect growth	36.9
June 20	3 inches	Many onion-like leaves. Erect	44.6
24	4 inches	Many onion-like leaves. Few panicle deformities [3]	41.8
27	4½ inches	Few onion-like leaves. Many panicle deformities	34.8 [2]
30	5 inches	Trace of onion-like leaves. Many panicle deformities, severe lodging	39.3 [2]
July 4	7 inches	Severe lodging. Panicle deformity	35.2 [2]
8	9 inches	Severe lodging. Few panicle deformities	30.2 [2]
11	12 inches	Severe lodging	40.0
14	13 inches	Only slight lodging	44.6
July 18	16 inches	Normal	40.7
21	10 days before heading	Normal	46.6
25	7 days before heading	Normal	48.3
29	3 days before heading	Normal	46.7
Aug. 1	Heading	Normal	46.0
5	4 days after heading	Normal	45.5
8	7 days after heading	Normal	42.4
11	10 days after heading	Normal	47.8
Average yield of check plots			46.3
L.S.D. at 5 per cent level			11.08

[1] 5 and 9 days after seeding.
[2] Difference significant at 5 per cent level.
[3] Clustering of the panicle, non-split glumes, blasting, basal sterility.

The Effect of 2,4-D on Oats in the Field

In 1950 and 1951 Ajax oats was treated in the field with an ester formulation of 2,4-D at 8 ounces pure acid per acre. Treatments were made at 3-day intervals beginning before emergence and continuing until after heading. The pertinent data obtained are presented in Tables 5 and 6.

The occurrence of abnormalities paralleled closely those reported for the greenhouse study. Onion-like leaves resulted from treatment within 1 to 16 days after emergence in both years. This was followed by a short period when panicle abnormalities were induced.

Comparing the sensitivity of oats and barley it will be seen that oats is most susceptible at stages intermediate between the two most susceptible stages in barley. The difference can be accounted for when a comparison is made between the respective developmental processes in the two cereal crops. The production of leaf initials takes place over a much longer period in oats than in barley. Therefore panicle differentiation in oats takes place at a much later period than does spike differentiation in barley. Consequently damage to the oat panicle would occur later than damage to

TABLE 6.—EFFECT OF TREATMENT WITH 2,4-D (BUTYL ESTER) ON OATS. TREATMENTS MADE IN 1951 AT 3-DAY INTERVALS (BEGINNING AT PRE-EMERGENCE AND CONTINUING PAST THE HEADING STAGE). CHEMICAL APPLIED AT THE RATE OF 8 OZ. ACID EQUIVALENT PER ACRE

Date application	Stage of growth	Abnormalities	Yield bu./ac.
May 14	Pre-emergence [1]	Normal	130.8
17	Pre-emergence [1]	Normal	121.8
21	1 inch, 1 leaf	Few onion-like leaves	119.8
25	3 inches, 2 leaves	Onion-like leaves. Erect growth	130.0
May 30	4 inches, 3 leaves	Onion-like leaves. Erect growth	119.4
June 2	5 inches, 3 leaves	Onion-like leaves. A few panicle deformities [3]	118.8
5	6 inches, 4 leaves	Onion-like leaves. Panicle deformities	117.6
9	7 inches, 4 leaves	Panicle deformities, badly lodged	88.8 [2]
June 12	9 inches, 4-5 leaves	Panicle deformities. Lodged badly	85.0 [2]
15	10 inches, 5-6 leaves	Badly lodged	109.0
19	13 inches, 6 leaves	Slightly lodged	103.4
22	15 inches, 6-7 leaves	Normal	107.7
June 27	13 days before heading	Normal	123.9
30	10 days before heading	Normal	131.8
July 3	7 days before heading	Normal	112.3
6	4 days before heading	Normal	113.2
July 10	Heading	Normal	130.1
13	3 days after heading	Normal	117.6
16	6 days after heading	Normal	118.5
19	9 days after heading	Normal	118.8
Average yield of check plots			124.0
L.S.D. at 5 per cent level			21.52

[1] 4 and 7 days after seeding.
[2] Difference significant at 5 per cent level.
[3] Clustering of the panicle, non-split glumes, blasting, basal sterility.

the barley spike. Following the differentiation of the barley spike there is a period when 2,4-D causes little or no damage. This period is followed by the differentiation of the reproductive parts during which period 2,4-D causes a high degree of sterility. In oats as in barley sterility is induced by treating when the reproductive parts are differentiating, but this differentiation takes place sooner in relation to inflorescence differentiation than in barley. Soon after panicle differentiation in oats the anthers are formed and consequently the sterility caused by 2,4-D takes place sooner in relation to heading in oats than in barley.

Yield reductions at early stages corresponding to the production of leaf initials were quite serious in the barley experiment. In oats the results were somewhat inconsistent as between the field and greenhouse. In the greenhouse study serious yield reductions occurred as a result of treatment at these stages, but in the field no significant yield reductions were recorded, even though the onion-like leaves were present. This may have been due to the heavier rate of 2,4-D used in the greenhouse and also to the shorter period between treatments in the greenhouse. Under the more widely spaced treatments in the field, there probably was an offsetting effect as between main culms and tillers, or between successive tillers. Moreover, the slower growing less succulent plants in the field were probably less susceptible than the more rapid growing lush plants in the greenhouse.

SUMMARY

Barley and oats were sprayed, in the greenhouse, with an aqueous solution of 2,4-D at 32 and 30 stages of growth, respectively, beginning before emergence and continuing at 1- or 2-day intervals until after heading. Photomicrographs were made at each spraying date to determine the stage of development of the growing point. In addition, oats was treated in the field at 20 stages of growth, at 3-day intervals between pre-emergence and post-heading.

In barley there were two critical periods when severe damage resulted from treatment. The first was a seedling period extending from emergence to the early 3-leaf stage. This damage took place during the period when leaf initials were forming and the tiny spike was differentiating in the growing point. Conspicuous leaf deformities characterized the early part of this period, and spike or spikelet deformities the latter part. The second susceptible period began when the plants were well advanced into the boot stage, 12-14 days before heading, and continued until just before spike emergence. A high degree of sterility was associated with the damage that took place at this time and occurred during the differentiation of anthers and stigma and early growth of the floral parts.

The incidence of abnormalities in oats corresponded with that in barley except that in oats leaf deformities appeared over a much longer period of time. The differentiation of anthers in oats, on the other hand, began much earlier in relation to inflorescence differentiation, than was the case with barley. Pistil differentiation occurred relatively later in oats than in barley. (See Figure 1.) These facts appear to account for the difference between oats and barley as regards the growth period during

FIGURE 1. Comparison of oats and barley as regards inception and duration of developmental stages and periods of susceptibility to treatment with 2,4-D.

which the greatest yield reduction is induced by treatment in the field. That period, in oats, has consistently been intermediate between the two highly susceptible periods in barley.

In the case of oats the greenhouse and field results were not consistent as regards severity of yield reduction resulting from treatment during the seedling stages. Severe reduction was recorded in the greenhouse for treatment at these stages, while little or no reduction was recorded in the field. The difference may have been due to the wider spacing of treatments in the field, to greater susceptibility associated with greater succulence of the plants in the greenhouse, and also to the heavier rate of application used in the greenhouse (12 oz. vs. 8 oz.).

REFERENCES

1. Andersen, S., and J. Hermansen. Effect of hormone derivatives on cultivated plants. Den Kgl. Veterinaer og Landbøhojskoles afdeling for Landbrugets Plantedyrkning. (Dept. for Agricultural Plant Culture of the Royal Veterinary and Agricultural High School.) Paper No. 26. 1950.
2. Bonnet, O. T. The development of the barley spike. J. Agr. Res. 51 : 451-457. 1935.
3. Bonnet, O. T. The development of the oat panicle. J. Agr. Res. 54 : 927-931. 1937.
4. Davidson, J. G. Effect of 2,4-D on spring sown oats when applied at two rates and at different growth stages. *In* Res. Rept., 8th Annual North Central Weed Control Conference, Oklahoma City, Okla. 1951.
5. Derscheid, L. A. Physiological and morphological responses of barley to 2,4-Dichlorophenoxyacetic acid. Plant Phy. 27 : 121-134. 1952.
6. Friesen, H. A., and M. D. Harris. Reaction of oats to 2,4-D applied at various growth stages. *In* Res. Rept., 8th Annual North Central Weed Control Conference, Oklahoma City, Okla. 1951.
7. Friesen, Geo., P. J. Olson, and D. A. Brown. Reaction of oats to 2,4-D when applied at different stages of growth. *In* Res. Rept., 8th Annual North Central Weed Control Conference, Oklahoma City, Okla. 1951.
8. Leggett, H. W. The effect of 2,4-Dichlorophenoxyacetic acid sprayed on Larain oats at 3-day intervals. *In* Proceedings and Abstracts of the 5th Western Canadian Weed Control Conference, Vancouver, B.C. 1951.
9. Olson, P. J., S. Zalik, W. J. Breakey, and D. A. Brown. Sensitivity of wheat and barley at different stages of growth to treatment with 2,4-D. Agronomy J. 43 : 77-83. 1951.

FERTILITY STUDIES ON SOIL TYPES

III. PHOSPHORUS SUPPLY AND REQUIREMENT AS SHOWN BY GREENHOUSE STUDIES AND LABORATORY TESTS[1]

A. J. MacLean,[2] R. F. Bishop and L. E. Lutwick[3]

Canada Department of Agriculture, Ottawa

[Received for publication November 7, 1952]

ABSTRACT

Oats and alfalfa have been grown in the greenhouse on samples of surface soil taken from nine farms on each of ten soil types occurring in the Ottawa district. The effect of applied phosphorus, as shown by yields and the phosphorus content of the crops, is used as a basis for evaluating various laboratory methods for estimating the soil phosphorus available for plant growth.

The relative effect of applied phosphorus on the uptake of phosphorus varies significantly according to soil type.

The amounts of phosphorus extracted from surface soil samples, by five of the chemical procedures employed, varies significantly between soil types. The values obtained by three of these procedures increases with increasing clay content of the samples, whereas, with the other two procedures, higher values are obtained for the soils of lighter texture.

The correlation coefficients relating greenhouse results with those obtained by the Neubauer procedure and by four of the ten chemical procedures employed are highly significant.

INTRODUCTION

Some results obtained in field, greenhouse and laboratory studies with different soil types occurring in the vicinity of Ottawa, Ontario, were presented in a previous paper (10). In addition to gaining information on the phosphorus status of these soil types, one of the objectives in these studies was to correlate chemical tests for phosphorus with crop response to fertilizer phosphorus applied in greenhouse tests. In the present paper, the results obtained on this phase of the work will be presented.

Information relating to the correlation of soil tests with crop response to applied phosphorus on Canadian soils is very limited (2, 14). Literature citations pertaining to this type of work in the United States may be found in a recent report (11).

Composite samples of surface soil from cultivated fields of nine farms on each of ten soil types were used in the present study. The soils have been discussed in soil survey reports (6, 13). The physical and chemical composition of the particular soil samples under study has been described in a previous paper (1).

MATERIALS AND PROCEDURE

Samples of surface soil from three farms on each of ten soil types were obtained in the fall of 1946. Similar sets of samples were obtained from other farms on the same soil types in the fall of 1947 and the fall of 1948. For greenhouse tests, the soils were air-dried, passed through a sieve with one-half inch mesh, mixed, and placed in glazed gallon pots on a volume

[1]Presented before the Soils Section, Agricultural Institute of Canada, Ottawa, June 26, 1952. Joint contribution from the Division of Field Husbandry, Soils and Agricultural Engineering, Experimental Farms Service, and the Division of Chemistry, Science Service. Scientific Contribution No. 230, Division of Chemistry.

[2] Senior Agronomist, Division of Field Husbandry, Soils and Agricultural Engineering.

[3] Chemist and Assistant Chemist, respectively, Division of Chemistry.

basis. Samples of the air-dried soil were passed through a 2-mm. sieve and retained for analyses in the laboratory. Oats and alfalfa were seeded together in the fall of the year of sampling, and later thinned to seven oat plants and ten alfalfa plants per pot. Fertilizers were placed in the soil in a layer at a depth of two inches, oat seeds at a depth of one inch, and alfalfa seeds at a depth of one-half inch. The treatments, relevant to this discussion, were 4-0-10 and 4-10-10 fertilizers applied at the rate of 400 pounds per acre. The oats were harvested in the spring, and during the summer either three or four crops of alfalfa were harvested. There were six replications for oats and three for alfalfa, except in the tests conducted in 1946-47, where there were twelve replications for oats and nine for alfalfa. The yields of grain, straw and alfalfa were obtained for each pot. The per cent phosphorus in each of these materials, grown with and without applied phosphorus, was determined on composite samples from all replications. The materials were ground in a Wiley mill and the phosphorus content determined according to the method of King (8).

Estimates of so-called "available" soil phosphorus were made by the Neubauer method (18) and by a number of chemical methods utilizing extraction procedures employed by various workers (3, 4, 5, 7, 9, 12, 15, 16, 17). To determine phosphorus in the extracts, readings were made with a photoelectric colorimeter. Information relative to the extracting procedure for the methods employed is given in Appendix A.

RESULTS AND DISCUSSION

Yields of Oats and Alfalfa in Greenhouse

The mean yields of grain, straw and alfalfa grown with and without phosphorus fertilizer are presented, for each soil type, in Table 1. The results indicate that under greenhouse conditions, application of phosphorus fertilizer tends to have less influence on the yield of oats and alfalfa on Uplands, Rubicon, Kars and Rideau soils than on samples from the other

TABLE 1.—EFFECT OF APPLIED PHOSPHORUS ON YIELD OF OATS AND ALFALFA IN THE GREENHOUSE

(Mean yield per pot for 9 tests on each soil type; fertilizer applied at 400 lb. per acre)

Soil type	Grain		Straw		Alfalfa (air-dry)	
	4-0-10	4-10-10	4-0-10	4-10-10	4-0-10	4-10-10
	gm.	gm.	gm.	gm.	gm.	gm.
Uplands sand	4.2	5.1	10.6	12.5	11.6	11.9
Rúbicon loamy sand	4.9	5.8	10.3	13.5	9.9	14.2
Kars gravelly sandy loam	7.4	7.5	15.7	16.8	23.7	27.0
Grenville loam	6.5	9.5	12.3	17.8	13.8	20.5
Manotick sandy loam	6.3	8.1	12.5	16.0	12.3	17.5
Castor silt loam	5.2	7.6	11.0	15.5	10.5	16.0
Osgoode loam	5.1	7.0	9.4	13.9	11.5	18.4
Carp clay loam	6.9	8.6	13.4	18.2	14.7	22.1
North Gower clay loam	6.3	9.0	12.0	16.9	13.2	21.8
Rideau clay	8.7	9.4	17.1	20.8	21.1	24.6
L.S.D. (0.05)*	1.2		1.7		2.3	

* Based on pooled (phosphorus × farms) interaction within each soil type.

soil types investigated. Where no phosphorus was applied, the samples of Rideau clay and Kars gravelly sandy loam produced the highest yields of oats and alfalfa.

Per Cent Phosphorus in Crops

The percentages of phosphorus in the grain, straw, and alfalfa crops grown with and without phosphorus fertilizer are presented as mean values for each soil type in Table 2. Application of phosphorus had no consistent effect on the percentage of phosphorus in the alfalfa crops grown on the different soils, although the phosphorus content of the oat crop was increased slightly on most of the soils as a result of the phosphorus treatment. The phosphorus content of the oat crop grown on Uplands, Kars, and Rideau soils, and of alfalfa grown on Kars soil, was relatively high as compared with the values obtained for these crops on the other soils. As already indicated, the effect of applied phosphorus on yield was relatively low on these three soils.

TABLE 2.—EFFECT OF APPLIED PHOSPHORUS ON THE PER CENT PHOSPHORUS (P) IN OATS AND ALFALFA GROWN IN THE GREENHOUSE

(Mean values, air-dry basis, for 9 tests on each soil type; fertilizers applied at 400 lb. per acre)

Soil type	Grain		Straw		Alfalfa	
	4-0-10	4-10-10	4-0-10	4-10-10	4-0-10	4-10-10
	%	%	%	%	%	%
Uplands sand	0.31	0.33	0.08	0.09	0.24	0.25
Rubicon loamy sand	0.29	0.31	0.04	0.04	0.24	0.25
Kars gravelly sandy loam	0.35	0.36	0.10	0.11	0.28	0.27
Grenville loam	0.27	0.28	0.03	0.03	0.20	0.17
Manotick sandy loam	0.27	0.30	0.03	0.04	0.22	0.22
Castor silt loam	0.29	0.30	0.02	0.03	0.20	0.23
Osgoode loam	0.30	0.31	0.03	0.02	0.21	0.21
Carp clay loam	0.26	0.32	0.02	0.04	0.18	0.16
North Gower clay loam	0.27	0.30	0.02	0.03	0.20	0.18
Rideau clay	0.32	0.37	0.07	0.08	0.22	0.22
Average	0.29	0.32	0.04	0.05	0.22	0.22
L.S.D. (0.05)*	0.03		0.01		0.03	

* Based on pooled (phosphorus × farms) interaction within each soil type, and applicable to differences between the mean values given for each soil type.

On the basis of the alfalfa harvested on the 90 soil samples, the higher the phosphorus content of the crop grown without phosphorus fertilizer, the lower was the increase in yield from applied phosphorus (Figure 1). The correlation coefficient (− 0.514) was significant at the 1 per cent level. These results lend support to the use of plant analysis as an aid in predicting crop response to phosphorus fertilization.

Phosphorus Removed by Crops

The amounts of phosphorus taken up by the oat and alfalfa crops as computed from yield and composition data served as a basis for appraising the phosphorus supply of the different soil samples. The average values for uptake of phosphorus by oats, by alfalfa, and by the two crops combined,

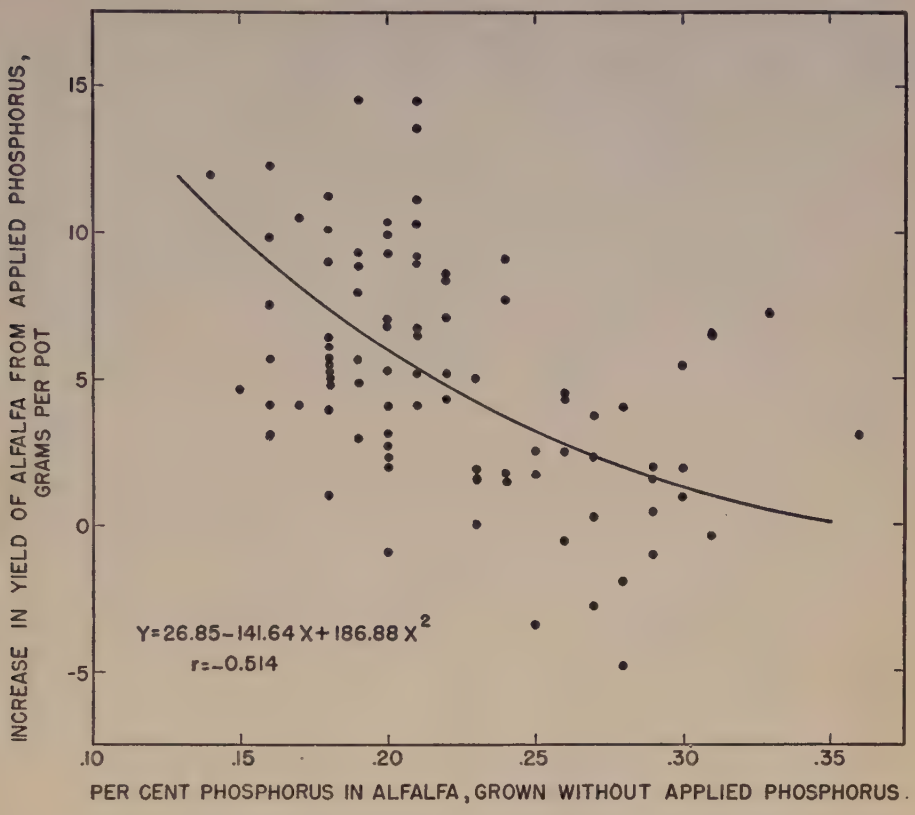


FIGURE 1. Relationship between the per cent phosphorus in alfalfa and the increase in yield of alfalfa from phosphorus fertilizer.

on the 4-0-10 and 4-10-10 treatments are presented for each soil type in Table 3. The uptake of phosphorus on the 4-0-10 treatment was expressed as a percentage of the uptake on the 4-10-10 treatment and is included in the table.

The analyses of variance in Table 4 indicate that applied phosphorus resulted in a highly significant increase in the uptake of phosphorus by oats and by alfalfa. The interaction of the phosphorus treatment on soil type was significant as measured by the uptake of phosphorus by the oat crop and on the basis of the combined uptake by oats and alfalfa. That is, the effect of applied phosphorus varied between soil types to a greater degree than between farms on the same soil type. The variations in the uptake of phosphorus by oats and by alfalfa between different soil types and between different farms on the same soil type were highly significant. However, the variation in uptake of phosphorus by the crops was greater between soil types than between farms on the same soil type, at the 1 per cent level.

With respect to phosphorus uptake on samples from individual soil types the following observations may be made on the basis of the data presented in Table 3. The response by oats and alfalfa to applied phos-

TABLE 3.—AMOUNTS OF PHOSPHORUS (P) REMOVED PER POT BY THE OAT AND ALFALFA CROPS IN THE GREENHOUSE
(Mean values for 9 tests on each soil type; 4-10-10 treatment contained 46 mgm. P per pot)

Soil type	Oats		Alfalfa		Oats + alfalfa		Uptake on 4-0-10 as per cent of uptake on 4-10-10		
	4-0-10	4-10-10	4-0-10	4-10-10	4-0-10	4-10-10	Oats	Alfalfa	Oats + alfalfa
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	%	%	%
Uplands sand	22.7	28.0	27.4	29.9	50.1	57.9	81	92	87
Rubicon loamy sand	20.0	25.0	25.7	34.7	45.7	59.7	80	74	77
Kars gravelly sandy loam	40.6	44.6	63.5	71.3	104.1	115.9	91	89	90
Grenville loam	21.0	31.1	28.0	40.7	49.0	71.8	68	69	68
Manotick sandy loam	20.7	30.2	30.4	39.5	51.2	69.7	69	77	73
Castor silt loam	17.6	27.0	21.7	34.9	39.3	61.9	65	62	63
Osgoode loam	18.8	24.8	25.1	36.8	43.9	61.6	76	68	71
Carp clay loam	20.0	32.9	27.4	34.7	47.4	67.6	61	79	70
North Gower clay loam	19.6	31.5	25.9	36.9	45.5	68.4	62	70	67
Rideau clay	39.1	49.8	48.1	54.2	87.2	104.0	79	89	84
L.S.D. (0.05) between treatments*	3.9		5.0		6.9		—	—	—
L.S.D. (0.05) between soils**	13.8		23.0		32.2		—	—	—

* Based on pooled (phosphorus × farms) interaction within each soil type.
** Based on pooled farm variance within each soil type.

TABLE 4.—ANALYSES OF VARIANCE OF DATA RELATING TO UPTAKE OF PHOSPHORUS BY OATS AND ALFALFA

Source of variation	D.F.	Oats		Alfalfa		Oats and alfalfa	
		M.S.	F	M.S.	F	M.S.	F
Soil type	9	1,224.30	72.27**	2,859.19	100.51**	7,510.75	140.91**
Farms within soil type	80	212.63	12.55**	592.91	20.84**	1,164.57	21.85**
Phosphorus	1	3,244.37	191.52**	3,693.76	129.85**	13,845.90	259.77**
Phosphorus × soil type	9	43.24	2.55*	49.91	1.76	115.14	2.16*
Phosphorus × farms (error)	80	16.94	—	28.45	—	53.30	—

* Significant at 0.05.
** Significant at 0.01.

phorus, on the basis of the uptake of phosphorus on the 4-0-10 treatment expressed as a percentage of that on the 4-10-10, was relatively low on the Kars, Uplands and Rideau samples as compared with that found for the other soils. The uptake of phosphorus by the crops on the Kars and Rideau soils was considerably higher than that obtained on the other soils. Although applied phosphorus increased the uptake of phosphorus by the crops to a lesser extent on Uplands sand than on the other soils, the uptake on the treated series of this soil type was relatively low.

SOIL PHOSPHORUS EXTRACTED BY VARIOUS PROCEDURES

Estimates of "available" soil phosphorus, as extracted by the procedures summarized in Appendix A, are presented as mean values for each soil type in Table 5. The extraction procedures of Peech and English, of Truog, and of Bray for "adsorbed" phosphorus were employed on the 90 soil samples, while with the other methods the number of samples varied from 13 to 58. Use of the K_2CO_3 and CO_2 procedures was confined to those samples with a neutral or alkaline reaction.

The variation in the amounts of phosphorus extracted from surface samples from different soil types by the Truog, Bray "adsorbed", Ruhnke, Ghani, and K_2CO_3 procedures exceeded the variation between samples from farms on the same soil type, at the 1 per cent level of significance. The F value reported for the Neubauer method approached significance at the 5 per cent level. The amounts of phosphorus extracted by the Truog, Ruhnke, and Ghani procedures increased with increasing clay content of the soils. The correlation coefficients, expressing the variation of clay content (1) with that of soil phosphorus extracted, were + 0.680 for the Truog values, + 0.642 for those obtained with the Ruhnke method (both significant at the 1 per cent level), and + 0.381 (significant at the 5 per cent level) where the Ghani method was employed. The amounts of phosphorus extracted by the K_2CO_3 and Bray "adsorbed" procedures tended to be of higher magnitude in the case of the lighter textured soils. The correlation coefficient (− 0.325), relating clay content and phosphorus extracted by the Bray "adsorbed" procedure was highly significant, and

TABLE 5.—MEAN VALUES FOR PHOSPHORUS (P) EXTRACTED BY VARIOUS PROCEDURES FROM SURFACE SAMPLES OF TEN SOIL TYPES

Soil type	Peech and English (9)*	Truog (9)*	Bray "adsorbed" (9)*	Bray "acid-soluble" (3)*	Ruhnke (3)*	Quebec (3)*	Ghani (3)*	Egner (3)*	K_2CO_3 (1-9)*	CO_2 (1-3)*	Neubauer (6)*
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Uplands sand	4.0	37	70	66	37	27	62	21	74	—	16
Rubicon loamy sand	5.5	57	15	45	69	31	159	25	74	1.29	12
Kars gravelly sand loam	6.0	68	25	46	87	46	148	25	140	2.04	20
Grenville loam	3.0	46	3	17	84	21	130	12	48	1.14	10
Manotick sandy loam	2.5	68	11	32	69	26	150	19	46	—	10
Castor silt loam	8.0	126	7	61	159	37	413	32	57	2.12	11
Osgoode loam	5.0	126	5	62	234	36	434	16	39	1.23	8
Carp clay loam	3.5	145	4	47	217	39	355	20	58	1.23	12
North Gower clay loam	5.0	179	4	49	265	44	407	18	45	1.05	10
Rideau clay	3.0	206	12	63	245	61	308	23	—	—	17
Average, for all samples	4.5	106	16	49	147	37	257	21	58	1.31	12
Total number of samples	90.0	90	90	30	30	30	30	30	39	13.00	58
M.S. between soil type	25.28	32,104.21	3,702.74	730.83	22,640.37	413.80	59,177.01	92.19	3,183.58	0.25	85.41
M.S. between farms on the same soil type	17.28	2,029.60	366.24	527.72	3,281.71	435.36	6,302.72	339.02	378.86	0.30	44.76
F value	1.46	15.82**	10.11**	1.38	6.90**	—	9.39**	—	8.40**	—	1.91

* Number of samples representing soil type. The results of the Neubauer procedure on Rubicon loamy sand are based on 4 samples only.
** Significant at 0.01.

the corresponding value (-0.300) for the K_2CO_3 method, approached significance at the 5 per cent level. These results indicate that the amount of phosphorus extracted by a number of the procedures employed may be expected to vary with soil properties associated with soil type.

RELATIONSHIP OF "AVAILABLE" SOIL PHOSPHORUS AND GREENHOUSE RESULTS

The uptake of phosphorus by oats and alfalfa on the 4-0-10 treatment, expressed as a percentage of the uptake on the 4-10-10, was used as a basis for appraising the various laboratory methods employed for estimating soil phosphorus. The relationships between the greenhouse results and the amounts of phosphorus extracted from the samples by three of these procedures, namely the K_2CO_3, Bray "adsorbed", and Peech and English, are shown in Figures 2, 3, and 4*, respectively.

* For the sake of brevity, the different soils are identified in the graphs by the soil series name.

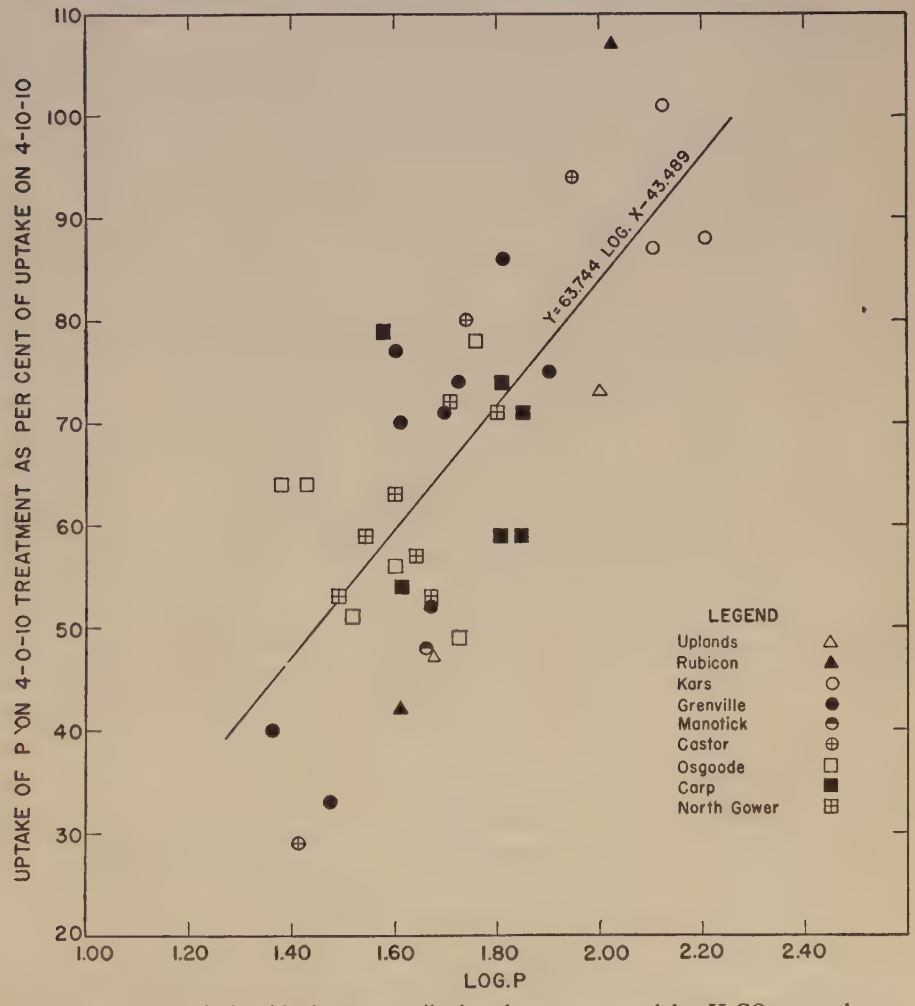


FIGURE 2. Relationship between soil phosphorus extracted by K_2CO_3 procedure and greenhouse results.

TABLE 6.—RELATIONSHIP OF SOIL PHOSPHORUS VALUES AND UPTAKE OF PHOSPHORUS BY THE CROPS ON THE 4-0-10 TREATMENT EXPRESSED AS A PERCENTAGE OF THE UPTAKE ON THE 4-10-10

Extraction procedure	D.F.	Correlation coefficient
Peech and English	88	+ 0.358**
Truog	88	+ 0.023
Bray "adsorbed"	88	+ 0.456**
Bray "acid soluble"	28	+ 0.317
Ruhnke	28	− 0.091
Quebec	28	+ 0.388*
Ghani	28	− 0.297
Egner	28	+ 0.432*
K_2CO_3	37	+ 0.693**
CO_2	11	+ 0.724**
Neubauer	56	+ 0.512**

* Significant at 0.05.
** Significant at 0.01.

TABLE 7.—RELATIONSHIP OF SOIL PHOSPHORUS VALUES AND UPTAKE OF PHOSPHORUS BY THE CROPS ON THE 4-0-10 TREATMENT AS A PERCENTAGE OF THE UPTAKE ON 4-10-10, FOR INDIVIDUAL SOIL TYPES

Soil type	D.F.	Correlation coefficients		
		Peech and English	Truog	Bray "adsorbed"
Uplands sand	7	+ 0.549	+ 0.519	+ 0.602
Rubicon loamy sand	7	+ 0.614	+ 0.736*	+ 0.701*
Kars gravelly sandy loam	7	+ 0.736*	+ 0.287	− 0.191
Grenville loam	7	+ 0.656	+ 0.488	+ 0.329
Manotick sandy loam	7	+ 0.655	+ 0.006	+ 0.793*
Castor silt loam	7	+ 0.646	− 0.181	+ 0.509
Osgoode loam	7	+ 0.134	− 0.007	+ 0.863**
Carp clay loam	7	+ 0.041	− 0.194	+ 0.658
North Gower clay loam	7	+ 0.239	− 0.557	+ 0.536
Rideau clay	7	+ 0.602	+ 0.216	+ 0.901**

* Significant at 0.05.
** Significant at 0.01.

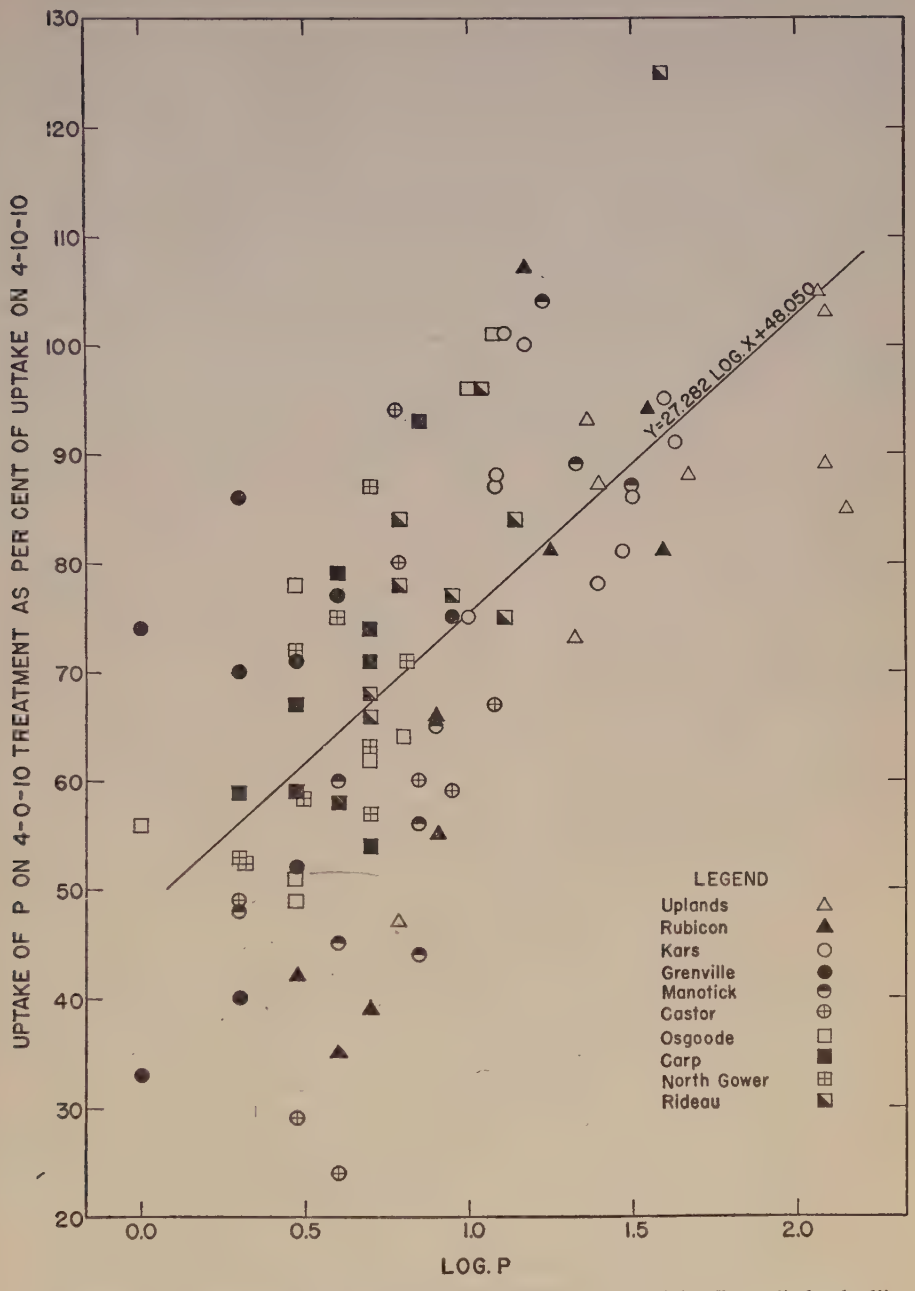


FIGURE 3. Relationship between soil phosphorus extracted by Bray "adsorbed" procedure and greenhouse results.

The correlation coefficients in Table 6, computed without regard to soil type, indicate that some of the methods for extracting phosphorus from the samples investigated were more satisfactory than others. The amounts of phosphorus extracted by the CO_2 and K_2CO_3 procedures, used only on neutral to alkaline samples, were highly correlated with the uptake of

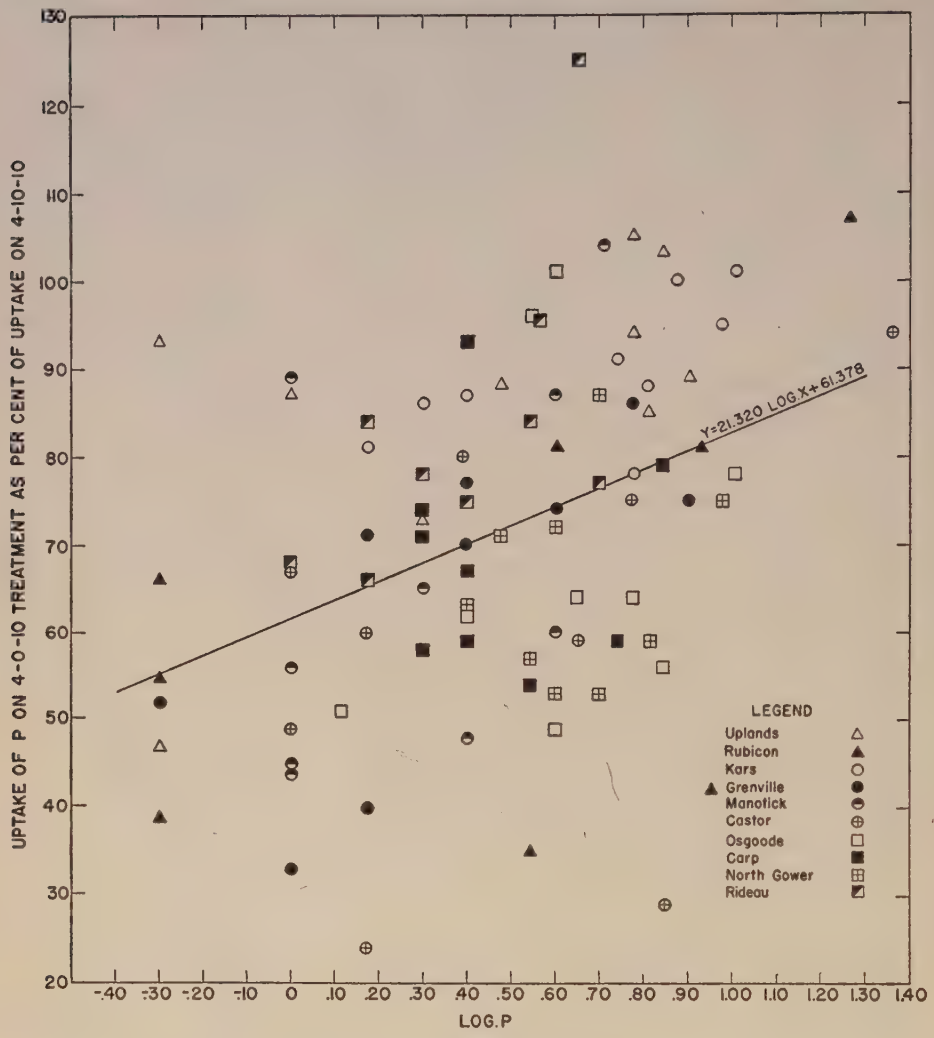


FIGURE 4. Relationship between soil phosphorus extracted by Peech and English procedure and greenhouse results.

phosphorus by the crops on the 4-0-10 treatment expressed as a percentage of the uptake on the 4-10-10. The corresponding correlation coefficients for the Neubauer, Bray "adsorbed", and Peech and English procedures were highly significant, whereas those for the Egner and Quebec procedures were significant at the 5 per cent level. The K_2CO_3 and CO_2 procedures employed on neutral to alkaline samples, and Bray "adsorbed" procedure, which was carried out on all samples, were the most satisfactory chemical methods for estimating phosphorus in the soils under study. With respect to the Bray procedures, on the basis of the 30 samples for which data are available, there was evidence in favour of combining the values designated as "adsorbed" and "acid-soluble" phosphorus for a particular sample. Thus, in relation to greenhouse results, a highly significant correlation coefficient (+ 0.475) was found for "adsorbed" plus "acid-soluble" phosphorus as

compared with + 0.318 for "adsorbed" phosphorus and + 0.317 for "acid-soluble" phosphorus, in the same samples. Bray (3) suggests that the sum of the "adsorbed" and "acid-soluble" forms of phosphorus gives the best correlation with the crop response to added phosphorus for corn belt crops.

Since nine phosphorus values were available for each soil type, in the case of the Peech and English, Truog and Bray "adsorbed" procedures, it was feasible to calculate the correlation coefficients relating the soil test values by these methods and greenhouse results, on the basis of individual soil types. The correlation coefficients in Table 7 present some evidence that a particular method may be more satisfactory for samples from some soil types than from others. For instance, with the Peech and English procedure, the correlation coefficients for Osgoode loam, Carp clay loam, and North Gower clay loam were relatively low compared with those found for the other soils. No explanation for this is suggested. It is interesting to note, however, that these three soil types have certain features in common. Each is derived from limy lacustrine material and possesses either poor or imperfect drainage. Although the results for the Truog procedure showed no correlation with greenhouse results on the basis of all samples, a significant correlation coefficient was obtained for this method in the case of Rubicon loamy sand. The correlation coefficients for the Bray "adsorbed" procedure varied somewhat with soil type but an appreciable degree of correlation was obtained on all samples excepting those from Kars gravelly sandy loam and Grenville loam. These results indicate that the nature of the soil should be considered when selecting a method for estimating soil phosphorus.

Although the results obtained with a number of the laboratory procedures were correlated with greenhouse results, it is evident from Figures 2, 3 and 4 that the values for the individual soil samples deviate considerably from the line that best fits the data.

ACKNOWLEDGMENTS

The authors wish to express appreciation to G. B. Oakland, Head, Biometric Unit, Science Service, Department of Agriculture, Ottawa, for several suggestions with regard to statistical treatment of data.

REFERENCES

1. Atkinson, H. J., G. R. Giles, and A. J. MacLean. Fertility studies on soil types: II. The physical and chemical composition of soils from Carleton and Grenville Counties in Ontario. Can. J. of Agr. Sci. 33 : 116-124. 1953.
2. Atkinson, H. J., P. O. Ripley, and L. M. Patry. Rapid soil tests on some Carleton County soils. Sci. Agr. 25 : 231-252. 1945.
3. Bray, Roger, and L. T. Kurtz. Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 59 : 39-45. 1945.
4. Egner, Hans. Method Att Bestamma Lattloslig Fosforsyra I. Akerjord Meddelande Nr 425 fran Centralanstalten for forsoksvasendet pa jordbruksomradet Avdelningen for lantbrukskemi Nr 51. Stockholm, 1932.
5. Ghani, M. O. The use of 8-hydroxy quinoline as a means of blocking active iron and aluminum in the determination of available phosphoric acid of soils by dilute acid extraction. Ind. J. Agr. Sci. 13 : 562-565. 1943.
6. Hills, G. A., N. R. Richards, and F. F. Morwick. Soil survey of Carleton County. Report No. 7, Ontario Soil Survey, Guelph, Ont. March, 1944.

7. Hockensmith, R. D., R. Gardner, and J. Goodwin. Comparison of methods for estimating available phosphorus in alkaline calcareous soils. Colo. Agr. Exp. Sta. Tech. Bull 2. 1933.
8. King, E. J. The colorimetric determination of phosphorus. Biochem. J. 26 : 292-297. 1932.
9. Lohse, H. W., and G. N. Ruhnke. Studies on readily soluble phosphate in soils: 1. Extraction of readily soluble phosphate from soils by means of dilute acid potassium sulfate ($KHSO_4$). Soil Sci. 35 : 437-457. 1933.
10. MacLean, A. J., *et al.* Fertility studies on soil types: 1. Some observations on Carleton County investigations. Sci. Agr. 29 : 1-12. 1949.
11. Nelson, W. L., *et al.* Soil testing in the United States. Report of the Soil Test Work Group of the National Soil and Fertilizer Research Committee. Dec. 1951.
12. Peech, M., and L. English. Rapid microchemical soil tests. Soil Sci. 57 : 167-195. 1944.
13. Richards, N. R., B. C. Matthews, and F. F. Morwick. Soil survey of Grenville County. Report No. 12, Ontario Soil Survey, Guelph, Ont. Dec., 1949.
14. Ruhnke, G. N., C. P. Rivaz, and W. T. Ewen. A comparative study of rapid chemical tests and Neubauer analyses on some typical Southern Ontario soils. Sci. Agr. 19 : 199-209. 1938.
15. Smith, V. T. An evaluation of the carbon dioxide method of determining available phosphoric acid in high lime soils. J. Amer. Soc. Agron. 40 : 1045-1046. 1948.
16. Truog, E. The determination of the readily available phosphorus of soils. J. Amer. Soc. Agron. 22 : 874-882. 1930.
17. Wrenshall, C. L., and R. R. McKibbin. A comparison of some methods used in extracting soil phosphates, with a proposed new method. J. Amer. Soc. Agron. 27 : 511-518. 1935.
18. Wright, L. E., *et al.* Chemical methods of soil analysis. Div. of Chemistry, Science Service, Canada Dept. of Agriculture, Ottawa. Jan., 1949.

APPENDIX A

SUMMARY OF EXTRACTION PROCEDURES USED IN THE ESTIMATION OF "AVAILABLE" PHOSPHORUS

Designation	Extractant	Ratio Soil (gm.) / Extractant (ml.)	Extraction time
Peech and English (12)	NaAc + HAc (pH 4.85)	10 : 50	30 minutes
Truog (16)	NH_4HSO_4 (pH 3.00)	2 : 400	30 minutes
Bray "adsorbed" (3)	HCl (0.025N) + NH_4F (0.03N)	1 : 7	60 seconds
Bray "adsorbed" + "acid-soluble" (3)	HCl (0.1N) + NH_4F (0.03N)	1 : 7	40 seconds
Bray "acid-soluble" (3)	By difference	——	——
Ruhnke (9)	$KHSO_4$ (pH 2.00)	2 : 400	5 minutes
Quebec (17)	Ca $(HSO_4)_2$ (pH 3.00)	2 : 400	30 minutes
Ghani (5)	HAc (0.5N) (pH 2.60)	2 : 400	60 minutes
Egner (4)	Ca-lactate + HCl (pH 3.70)	5 : 250	2 hours
K_2CO_3 (7)	K_2CO_3 (1% solution)	2 : 150	1 hour
CO_2 (15)	CO_2 bubbled into suspension	10 : 100	20 minutes
Neubauer (18)	Rye seedlings	——	16 days

* Numbers in brackets refer to literature cited.

A COMPARISON OF CERTAIN BREEDS OF RANGE SHEEP UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

S. B. Slen[2], F. Whiting[2], H. F. Peters[3] and W. N. MacNaughton[4]

Experimental Station, Lethbridge, Alta., and Range Experiment Station, Manyberries, Alta.

[Received for publication November 19, 1952]

ABSTRACT

A 3-year study is described in which the productivity of the Canadian Corriedale and the Romnelet breeds of sheep were compared under the different environmental conditions at the Experimental Stations at Lethbridge and Manyberries, Alberta. At Lethbridge, a group of Rambouillets also was included in the study.

There were no differences in clean wool production between the Canadian Corriedales and the Romnelets. The Rambouillet produced significantly more wool than the other two breeds. Station differences in wool production were significant.

At both Stations the Canadian Corriedale produced lambs that were significantly heavier than those from the Romnelet, but no Station differences were observed. Weaning weights showed no differences between these two breeds but Station differences were highly significant.

There were no significant differences between breeds in average daily gains, efficiency of feed utilization, or dressing percentage when fed a fattening ration. The Rambouillet had a significantly lower carcass grade than the Corriedale or Romnelet.

For the past 30 years the Experimental Farms Service has been involved in a sheep-breeding program designed to develop or improve a breed or breeds of sheep that would be adapted to the range conditions of Western Canada. To the present time two "breeds" (unregistered) have been developed for this purpose, namely, the Canadian Corriedale and the Romnelet, and the Rambouillet has undergone considerable improvement in body conformation and wool covering.

The Canadian Corriedale was developed at the Experimental Station, Lethbridge, Alberta, from mating imported New Zealand Corriedale rams to Lincoln × Rambouillet ewes and to subsequent progeny in a grading-up program. This procedure was begun in 1919 and continued until 1933 when the flock was closed and an intense program of inbreeding was undertaken. Since 1946 mild inbreeding has been followed.

The Romnelet breeding project was begun in 1935 at the Range Experiment Station, Manyberries, Alberta, by crossing purebred Romney Marsh rams with Rambouillet ewes. These ewes and rams were used only for the original cross and the progeny have been maintained up to the present time with an equal amount of Romney and Rambouillet blood. A mild type of inbreeding has been practised since the inception of this project.

In addition to these two projects, the Experimental Stations at Lethbridge, Alberta, and Swift Current, Saskatchewan, and the University of Saskatchewan, Saskatoon, have been engaged in an improvement program of the Rambouillet sheep. Although this breed is fundamental

[1] Contribution from the Division of Animal Husbandry, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada.

[2] Wool Specialist and Animal Nutritionist, respectively, Experimental Station, Lethbridge, Alta.

[3] Superintendent, Range Experiment Station, Manyberries, Alta.

[4] Animal Geneticist, Experimental Station, Lethbridge, Alta., present address Experimental Farm, Brandon, Man.

to the western range sheep industry, it had certain undesirable characteristics which, if they could be eliminated, would result in a breed more suitable than a crossbred type. Consequently, these Stations have sought to improve body conformation, reduce wrinkling and face cover, and increase staple length and clean wool production in this breed.

Prior to 1948 the Canadian Corriedale and the Romnelet had been maintained at the Stations where they were developed and no direct comparison of their relative potentialities as breeds had been made. On the suggestion of the Western Sheep Improvement Committee* it was decided to test these sheep reciprocally under the environmental conditions at both Lethbridge and Manyberries. This test was begun in 1948.

The two ranges on which the sheep were maintained are located approximately 200 miles apart and are classed as "short-grass". At Manyberries the range is entirely undisturbed with the predominant species being grama grass (*Bouteloua gracilis*), blue-joint (*Agropyron smithii*), speargrass (*Stipa comata*), junegrass (*Koeleria cristata*) and Sandberg's bluegrass (*Poa secunda*). The range used by the Lethbridge flock (Sheep Substation, Scandia, Alberta) included a considerable amount of abandoned farm land which has been allowed to return to grass and also a portion which has been seeded to crested wheat grass for early spring range. On this range grama grass (*Bouteloua gracilis*), spear grass (*Stipa comata*), slender wheat grass (*Agropyron trachycaulum*), and sandgrass (*Calamovilfa longifolia*) predominate.

REVIEW OF LITERATURE

Phillips and Spencer (5) made two comparisons of the reactions of Southdown sheep to the environment at Beltsville, Maryland, and at Middlebury, Vermont. In one comparison a flock was transferred from Beltsville to Middlebury and returned to Beltsville after it failed to thrive. In another case weanling lambs were transferred from Middlebury to Beltsville and their performance was compared with that of animals born five years earlier and retained at Middlebury. In the first comparison ewes of all ages at Middlebury were inferior in body weight, lambing percentage, staple length, and fleece weight to those at Beltsville. No difference in average birth weight was obtained between the two locations. In the second set of comparisons there were no consistent differences in performance of animals at the two Stations. The authors pointed out the importance of variations in adaptability to environment in livestock production.

Rasmussen and Weir (6) studied the effect of breed on feedlot performance and carcass characteristics of the Romney-Rambouillet cross, the Canadian Corriedale, and the Rambouillet as determined by feeding trials and carcass tests. They reported no significant differences in rate of gains between the three groups, nor was there any difference in the efficiency of feed utilization. Considerable variability was observed in carcass

* An advisory committee set up by the Canada Department of Agriculture to assist those engaged in range sheep breeding in Western Canada.

quality although the Romney-Rambouillet cross and Corriedale were superior to the Rambouillet.

Karam, Chapman and Pope (2) studied the effects of type of birth, sex, year, flock, and sire on body weight at 25 weeks of age, daily gains from birth to market, daily gain on feed, type and condition of 437 lambs raised in farm flocks. These lambs were sired by 23 rams leased to 13 commercial lamb producers during 1944-47. Eight lambs sired by each ram were brought to the Wisconsin Experiment Station at an average age of 25 weeks and fed out in sire-progeny groups for approximately 11 weeks. These workers found that location, type of birth, sex, and year had highly significant effects on nearly all the characters studied, and suggested that adjustments for them should be made before comparing lambs from different sires under such conditions.

PROCEDURE

In 1948 two groups, each of 50 ewes and two rams, were selected from the Romnelet flock, one of which was transferred to Lethbridge and the other retained at Manyberries for the breed comparison project. Similarly, two groups were selected from the Canadian Corriedales, one of which was transferred to Manyberries and the other retained at Lethbridge. Fifty Rambouillet ewes and two rams from the Lethbridge Station flock also were placed in this breed comparison test. The age distribution in all groups was the same at the inception of the project and replacement ewe lambs were obtained from within these groups each year. These sheep were maintained in the Station flocks under normal range conditions and although every effort was made to treat the two flocks similarly it was felt that those at Manyberries were kept on a winter ration of higher nutritive value.

Each year all ewes were bred so that lambing commenced during the first week in April. All male lambs were castrated within two weeks after birth and every effort was made to raise as many twin lambs as the milk supply and body condition of the ewe would permit.

Lamb production was studied on the basis of birth weights and weaning weights. Wool production was compared on the basis of raw and clean fleece weight, fibre thickness, staple length, and percentage yield of clean wool. All lambs from this project, with the exception of those kept for replacements, were fattened for market each year at the Experimental Station, Lethbridge (see Figure 1). A standard fattening ration consisting of hay and a grain mixture was fed. The lambs were fed until considered to be good choice and of a desirable market weight (100-105 pounds), and then were shipped for slaughter. Data were collected on average daily gains, feed consumption, dressing percentage, and carcass grades.

The data have been analysed for the period of 1948 to 1951, inclusive.

RESULTS AND DISCUSSION

Wool Production

A summary of the average wool production for the five groups at Lethbridge and Manyberries during 1948-51 is shown in Table 1.

TABLE 1.—AVERAGE WOOL PRODUCTION OF CANADIAN CORRIEDALE, ROMNELET, AND RAMBOUILLET EWES AT THE TWO STATIONS, 1948-51

	No. of fleeces	Raw fleece weight	Clean fleece weight	Percentage yield clean wool	Staple length	Fibre thickness
		lb.	lb.		mm.	μ
Lethbridge—						
Can. Corriedale	151	9.9	4.6	46.6[5]	95.0	25.7[5]
Romnelet	140	9.5[1]	4.7	49.7	92.6[2]	28.1
Rambouillet	146	11.5	4.9[3]	42.8[4]	77.4[4]	21.6[4]
Manyberries—						
Can. Corriedale	145	11.3	5.3	46.8[5]	106.5	26.4[5]
Romnelet	147	10.6[2]	5.3	49.9	98.1[2]	29.1
Stations Combined—						
Can. Corriedale	296	10.5	4.9	46.7[5]	100.7	26.0[5]
Romnelet	287	10.1[2]	5.0	49.8	95.4[2]	28.7

Average Fleece Weights by Years

	Canadian Corriedale		Romnelet		Rambouillet	
	Raw weight	Clean weight	Raw weight	Clean weight	Raw weight	Clean weight
	lb.	lb.	lb.	lb.	lb.	lb.
1949	10.6	4.9	10.2	5.0	11.3	4.7
1950	10.8	4.8	10.3	4.9	11.8	4.9
1951	10.4	5.0	9.8	5.0	11.5	5.1

[1] Significantly less than Can. Corriedale and Rambouillet ($p \leqq 0.01$).
[2] Significantly less than Can. Corriedale ($p \leqq 0.01$).
[3] Significantly greater than Can. Corriedale and Romnelet ($p \leqq 0.01$).
[4] Significantly less than Can. Corriedale and Romnelet ($p \leqq 0.01$).
[5] Significantly less than Romnelet ($p \leqq 0.01$).

It was found that the Canadian Corriedale produced significantly more raw wool than the Romnelet at both Stations. Actually, the difference between breeds was not important because the Romnelet consistently had a higher percentage yield of clean wool with the result that there was no difference in clean wool production. However, it is important to note that the sheep maintained at Manyberries produced 1.0 and 0.6 pound more raw and clean wool, respectively, than those at Lethbridge in spite of the fact they were handled quite similarly. This must be attributed in the main to nutritional differences between the two types of ranges. This is in agreement with the results of Phillips and Spencer (5) using Southdown sheep. When compared with the Rambouillet these two breeds grew significantly less ($p \leqq 0.01$) raw and clean wool although they had a significantly higher ($p \leqq 0.01$) percentage yield. Year differences were found to be highly significant in raw fleece weights but not in clean weights.

TABLE 2.—AVERAGE BIRTH WEIGHTS OF LAMBS AND LAMBING PERCENTAGES AS INFLUENCED BY BREED AND LOCATION, 1948-51

	Single lambs				Twin lambs				Lambing percentage[7]
	Male		Female		Male		Female		
	No. of lambs	Average birth weight	No. of lambs	Average birth weight	No. of lambs	Average birth weight	No. of lambs	Average birth weight	
		lb.		lb.		lb.		lb.	
Lethbridge—									
Canadian Corriedale	49	10.7	37	10.2	56	8.7	50	8.1	131.5
Romnelet	40	10.3[1]	40	9.0[2]	54	8.4	47	8.0	129.7
Rambouillet	39	11.1	51	10.4	33	8.7	60	8.4	127.9
Manyberries—									
Canadian Corriedale	46	10.3	43	10.0	44	9.2	53	8.4	128.7
Romnelet	46	9.8	36	9.2[3]	60	8.5[3]	59	7.6[4]	139.3
Stations Combined—									
Canadian Corriedale	95	10.5	80	10.1	100	8.9	103	8.3	130.1
Romnelet	86	10.0[3]	76	9.1[4]	114	8.4[3]	106	7.8[3]	134.5

Average Birth Weights by Years

	Canadian Corriedale	Romnelet	Rambouillet
1949	9.6	8.7	9.4[6]
1950	9.1[5]	8.6	9.3[6]
1951	9.5	8.9	10.0
Average weight (1949-51)	9.4	8.8[2]	9.6

[1] Significantly lighter than the Rambouillet ($p \leqq 0.05$).
[2] Significantly lighter than the Rambouillet and Canadian Corriedale ($p \leqq 0.01$).
[3] Significantly lighter than the Canadian Corriedale ($p \leqq 0.05$).
[4] Significantly lighter than the Canadian Corriedale ($p \leqq 0.01$).
[5] Significantly lighter than 1949 and 1951 ($p \leqq 0.05$).
[6] Significantly lighter than 1951 $\leqq$ (p 0.05).
[7] Based on number of births and number of ewes bred.

Highly significant year × location interactions were obtained for both raw and clean fleece weights.

At both Stations the Canadian Corriedale grew a significantly longer staple of wool than the Romnelet during a 12-month period. Station and year differences were highly significant as were year × location interactions. At Lethbridge the Rambouillet produced a considerably shorter staple of wool than the other two breeds.

With respect to average fibre thickness it was found that the Rambouillet was the finest (70s) and the Romnelet the coarsest (56s). The two breeds at Manyberries grew coarser-fibred fleeces than those in the Lethbridge flock indicating marked Station differences.

Since location differences were highly significant for all factors analysed, except percentage yield of clean wool, the data suggest that environment (including plane of nutrition) may be of greater importance than breed differences in influencing wool production.

Birth Weights of Lambs

The data relative to average birth weights of lambs from Manyberries and Lethbridge are presented in Table 2.

An analysis of variance indicated that there were highly significant differences in birth weights of lambs at both Stations due to breed, with the Canadian Corriedale averaging 0.6 pound heavier than the Romnelet. There were no significant differences in average birth weights of lambs between the Rambouillet and the Corriedale at Lethbridge but the Romnelet produced single lambs that were significantly lighter than the other two breeds. Female single lambs were approximately 0.7 pound lighter than single males ($p \leqq 0.01$) and twin females were approximately 0.6 pound lighter than twin males ($p \leqq 0.01$). Single lambs averaged 1.7 pounds heavier than twins. McLean (3) in an inheritance study of birth weight and growth rate found that single lambs were one to two pounds heavier at birth than twins, females were $\frac{1}{4}$ to $\frac{3}{4}$ pound lighter than males. Nelson and Venkatachalam (4) reported that birth weights of females were 5 per cent lighter than males, and single lambs were 22 per cent heavier than twins.

Significant differences in birth weights between years were obtained, presumably due to variations in the amount of feed available on the winter range and the degree of supplementation.

There were no significant differences between the lambing percentages of the three breeds at Lethbridge or the Canadian Corriedales at Manyberries. The lambing percentage of the Romnelets at Manyberries was somewhat, though not significantly, higher than that of the other breeds.

Weaning Weights

The average age at weaning was 120.1 days. The weight of each lamb was adjusted to 120 days of age by the analysis of covariance (7), using the error regression coefficient. Separate regression coefficients were determined for singles, twins, and twins raised as singles. These data are shown in Table 3.

TABLE 3.—AVERAGE WEANING WEIGHTS OF LAMBS FROM LETHBRIDGE AND MANYBERRIES (120 DAYS OF AGE), 1948-51

	Singles		Twins raised single		Twins		Pounds of lamb weaned per ewe lambed
	Number	Weight	Number	Weight	Number	Weight	
		lb.		lb.		lb.	
Lethbridge—							
Canadian Corriedale	76	63.2	31	63.2	39	55.7	67.0
Romnelet	76	65.8	30	63.6	36	55.4	67.7
Rambouillet	83	73.3[1]	30	65.1	26	60.2[1]	76.6
Manyberries—							
Canadian Corriedale	75	70.8	18	70.2	53	58.6	71.1
Romnelet	76	71.3	14	68.8	94	59.4	81.0
Stations Combined—							
Canadian Corriedale	151	67.0	49	65.8	92	57.4	69.0
Romnelet	152	68.6	44	65.3	130	58.3	74.4
Canadian Corriedale and Romnelets Combined—							
Lethbridge	152	64.5	61	63.4	75	55.5	67.4
Manyberries	151	71.1[2]	32	69.6[2]	147	59.1[2]	76.0
All males	194	69.9	55	66.1	127	60.3[3]	
All females	192	68.1	68	64.9	121	55.9	

[1] Significantly heavier than the other breeds at Lethbridge ($p \leqq 0.01$).
[2] Significantly heavier than lambs raised at Lethbridge ($p \leqq 0.01$).
[3] Significantly heavier than females ($p \leqq 0.01$).

There were no significant differences in weaning weights between the Romnelet and Corriedale lambs at either Lethbridge or Manyberries when adjusted to a constant age. At Lethbridge the Rambouillet weaned significantly heavier lambs than the other two breeds. Single lambs and twins raised as singles averaged 10 pounds heavier than twins, while sex differences were obtained only in the case of twins at weaning. McLean (3) found that at 100 days of age single lambs were six to seven pounds heavier than twins and males were three to four pounds heavier than females.

The differences in weaning weights between Stations and Station × year interactions were highly significant, again indicating that environmental factors must be considered when comparing various breeds of livestock. Hazel and Terrill (1), in analysing the effects of sex and year on weanling traits of lambs, found that these factors had a marked influence on weaning weight. Karam *et al.* (2) suggested that adjustments for environmental factors should be made before comparing lambs from different sires and locations.

There were no significant differences in pounds of lamb weaned per ewe lambed between breeds although the Romnelet at Manyberries averaged somewhat heavier than the Canadian Corriedale.

Feedlot Performance

Data on initial and final weights, gains, daily feed consumption, efficiency of feed utilization, dressing percentage, and carcass grades for all lambs fed in the feedlot are summarized in Table 4.

TABLE 4.—AVERAGE GAINS, FEED CONSUMPTION, AND CARCASS DATA FOR CANADIAN CORRIEDALE, ROMNELET, AND RAMBOUILLET LAMBS WHEN FED IN THE FEEDLOT, 1948-1951

	Lethbridge			Manyberries	
	Can. Corr.	Romnelet	Ramb-ouillet	Can. Corr.	Romnelet
No. of lambs weaned	146	142	139	146	184
No. of lambs fed	110	106	95	109	134
Av. number of days on feed	110	106	92	95	98
Av. initial weight (lb.)	69.9	71.4	80.9	78.7	76.7
Av. final weight (lb.)	96.7	96.3	102.3	100.9	99.4
Av. gain (lb.)	27.0	24.9	21.4	22.2	22.7
Av. daily gain (lb.)	0.25	0.24	0.23	0.23	0.23
Av. daily ration (lb.)—					
Hay	1.75	1.58	1.95	1.69	1.53
Grain mix	1.43	1.40	1.38	1.38	1.38
Av. feed consumption per 100 lb. gain—					
Hay	718	695	836	781	668
Grain mix	577	602	587	608	600
Av. T.D.N. consumption per 100 lb. gain	783	790	849	838	774
Av. dressing percentage	46.3	48.1	46.7	46.8	47.8
Av. carcass grade (%)—					
Choice	77.8	81.1	54.8	85.3	88.0
Good	17.6	17.0	30.1	13.8	12.0
Medium	4.6	1.9	15.1	0.9	0.0

There were no significant differences between the breeds from the two Stations relative to average daily gains, efficiency of feed utilization, or dressing percentages. However, the Rambouillet had a significantly lower carcass grade than the Canadian Corriedale or the Romnelet (see Figure 2). In a previous study Rasmussen and Weir (6) reported no significant differences in rate of gain, feed efficiency, or carcass grade between the Romney crossbreds and the Canadian Corriedales but both had a carcass grade superior to the Rambouillet.

Ewe Body Weights and Mortality

The average body weights of ewes at breeding and lambing together with the mortality are shown in Table 5.

TABLE 5.—AVERAGE BODY WEIGHTS (POUNDS) AND MORTALITY OF EWES, 1948-51

	No. of ewes	Body weight at breeding	Body weight at lambing[1]	No. of ewes died
		lb.	lb.	
Lethbridge—				
Canadian Corriedale	150	123.5	110.2	3
Romnelet	150	121.5	109.8	9
Rambouillet	150	134.6	125.7	5
Manyberries—				
Canadian Corriedale	150	126.8	119.1	4
Romnelet	150	132.5	122.9	2

[1] Weight taken within 24 hours after lambing.

It was found that the Canadian Corriedales were heavier during the 3-year period than the Romnelets at Lethbridge but the reverse occurred at Manyberries. This was to be expected as the sheep, of necessity, would undergo a period of acclimatization which would be reflected in their body weights. At Manyberries it was noted that after three years the original Canadian Corriedale had become acclimatized and ewes were slightly heavier than the original Romnelets. At Lethbridge the Canadian Corriedale and the Romnelet were approximately 10 pounds lighter than the Rambouillet.

Death losses were not serious in any of the breeds studied. Losses were found to be higher among the Romnelets transferred to Lethbridge than among the Corriedales moved to Manyberries. However, mortality was higher in the transferred groups than in those remaining at the parent Station.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of L. E. Meldrum and R. D. Clark who were Officers-in-Charge of the Sheep Substation, Scandia, Alberta, from May, 1949 to May, 1950 and May, 1950 to October, 1951, respectively; and K. McKenzie, formerly Assistant in Animal Husbandry at the Range Experiment Station, Manyberries, Alberta, for the collection of certain data used in this study.

FIGURE 1. Representative groups of Canadian Corriedale, Romnelet, and Rambouillet lambs (*from top to bottom*) being fattened at the Experimental Station, Lethbridge, during the winter of 1949-50.

FIGURE 2 Representative carcasses from the three breeds compared in this test. Note that the Canadian Corriedale (*left*) and the Romnelet (*right*) have a shorter and thicker type of carcass and have a better finish than that of the Rambouillet (*centre*).

REFERENCES

1. Hazel, L. N., and C. E. Terrill. Effects of some environmental factors on weanling traits in range Rambouillet lambs. J. An. Sci. 8 : 331-341. 1945.
2. Karam, H. A., A. B. Chapman, and A. L. Pope. Some of the sources of variation in body weight, rate of gain, type and condition of lambs raised under farm conditions. J. An. Sci. 8 : 606. (*Abstract*). 1949.
3. McLean, J. W. Progeny testing in sheep. The inheritance of birth weight, growth rate, and cannon bone length. Proc. 8th Ann. Conf., N.Z. Soc. An. Prod. 86-96. 1948.
4. Nelson, R. H., and G. Venkatachalam. Estimates of the heritability of birth weight and weaning weight of lambs. J. An. Sci. 8 : 607-608. (*Abstract*). 1949.
5. Phillips, R. W., and D. A. Spencer. Reactions of Southdown sheep to two environments. J. An. Sci. 7 : 41-49. 1948.
6. Rasmussen, K., and J. A. Weir. The effect of breeding on feedlot performance and carcass characteristics of lambs as determined by feeding trials and carcass tests. Sci. Agr. 21 : 153-166. 1940.
7. Snedecor, G. W. Statistical methods. Iowa State College Press, Amos, Iowa. 4th ed. 1946.

EFFECT OF 2,4-D ON THE NITRATE CONTENT OF FORAGE CROPS AND WEEDS

R. T. Berg[1] and L. W. McElroy[2]

Department of Animal Science, University of Alberta, Edmonton, Alta.

[Received for publication November 10, 1952]

ABSTRACT

Forage plants and weeds grown in the Edmonton district were treated with 2,4-D, and nitrate determinations were made on samples from treated and check plots using phenoldisulfonic acid. All samples of oats assayed contained high levels of nitrate but no evidence of an effect of treatment with 2,4-D on nitrate content was found in oats, brome grass, timothy, alfalfa, red clover, sweet clover or white Dutch clover. Relatively high concentrations of nitrate were found in Canada thistle, dandelion, lamb's quarters, redroot pigweed, Russian pigweed and Russian thistle. The nitrate content of Russian pigweed and Canada thistle samples taken from treated plots was significantly higher than that of samples of these weeds taken from check plots.

INTRODUCTION

According to Bradley, Eppson and Beath (2) the lower toxic limit of nitrate in cattle feeds may be in the vicinity of 1.5 per cent KNO_3 equivalent on a dry weight basis. In 1950 Stahler and Whitehead (8) reported nitrate levels in sugar beet leaves accidentally sprayed with 2,4-D averaging 20 times those in unsprayed leaves. On the basis of this observation, and the fact that a number of common weeds are often characterized by a high nitrate content even in the absence of treatment with herbicides, they stressed the need for information on the accumulation of nitrates in crop plants and weeds treated with 2,4-D. The results of a study initiated in 1951 to obtain data on the effect of 2,4-D treatment on the nitrate content of a number of forage plants and weeds grown in the Edmonton district are reported in the present paper.

EXPERIMENTAL

Assay Method

A number of methods of assay were compared. The one adopted for routine use was a modification of the phenoldisulfonic acid method described by Snell and Snell (7). The water extract of a 1-gram sample was decolorized with activated charcoal. Each batch of charcoal was tested to make certain it did not adsorb nitrates. The nitrate in the decolorized solution was determined with the aid of a Beckman spectrophotometer at a wave length of 408 millimicrons. The concentration of nitrate and nitrite nitrogen expressed as KNO_3 was calculated from a standard curve. The results obtained by this method were in agreement with those obtained by using the A.O.A.C. (1) procedure.

Assay Material

Assays were conducted on cuttings, 50-100 grams air dry weight, from 11 ft. × 11 ft. squares with a 9-ft. border between sprayed and unsprayed

[1] Lecturer in Animal Husbandry.
[2] Professor of Animal Science.

plots. In 1951 cuttings were taken 5 and 10 days after treatment, while in 1952 a number of samples were collected at 2, 5 and 10 days following treatment. In 1951 the plots of sweet clover, lamb's quarters, and Canada thistle were in flower when treated; in all other cases treatment was applied after the plants had made considerable growth but before they had reached the flowering stage.

RESULTS AND DISCUSSION

A summary of results is given in Table 1.

The level of nitrates in oats was high in samples from both the treated and the untreated plots. Analysis of variance of the concentration of KNO_3 in the oat samples showed no difference between 2,4-D treated and untreated plots. The average KNO_3 equivalent of all the oat samples was 3.62 per cent with a range of 2.42-4.98 per cent. Olsen and Whitehead (5) reported 20 oat hay samples averaging 3.15 per cent and ranging from 0.41 per cent to 5.99 per cent KNO_3 equivalent. The oat samples in the present study were taken from a rank growth on land which had in previous years been pastured by pigs. High levels of nitrate in the oats may have been caused by increased fertility of the soil attributable to the droppings from pigs. Mayo (4) and Eckerson (3) have reported increases in nitrate content of plants following fertilization with KNO_3 and $Ca(NO_3)_2$, respectively.

The remaining hay and forage crops sampled contained less than 0.25 per cent KNO_3 and there were no differences between 2,4-D treated and untreated plots.

From the data in Table 1 it appears that treatment with 2,4-D tended to result in appreciably higher levels of nitrate in Russian pigweed and Canada thistle. The results of analysis of variance (6) given in Table 2 show that the increase in nitrates obtained in treated samples of Russian pigweed is significant at the 1 per cent point. Similar calculations indicated that in 1952 the results for Canada thistle were significant at the 5 per cent level. None of the other differences was found to be statistically significant. The data presented in Table 2 also show that samples taken on the 2nd, 5th and 10th days following spraying contained essentially the same level of nitrates. Similar results were obtained for samples of other species of weeds cut 2 to 10 days after treatment.

Thorpe (9) reported lamb's quarters, pigweed and Russian thistle present as contaminants in hays causing "oat hay poisoning" in cattle. Samples of these weeds analysed in the present study contained high levels of nitrate. Nitrate levels in lamb's quarters were exceptionally high in the 1952 samples from both treated and untreated plots. These samples were collected from the same hog pasture from which the oat samples mentioned earlier were gathered.

Concentration of nitrates in stinkweed was extremely variable in both treated and untreated samples, with a range of 0.2 per cent to 3.3 per cent KNO_3 equivalent. The nitrate content of dandelion samples collected in 1952 was below the toxic level of 1.5 per cent suggested by Bradley *et al.* (2). Despite the fact that they were sprayed at the rate of 8 oz. of 2,4-D acid

TABLE 1.—NITRATE AND NITRITE NITROGEN EXPRESSED AS PER CENT KNO_3 OF DRY WEIGHT

	Treated				No treatment		
	2,4-D acid equivalent oz./acre	Number of samples	Mean	Range	Number of samples	Mean	Range
Cultivated Crops							
Alfalfa							
1951	4	4	0.16	0.11–0.21	4	0.12	0.10–0.16
1952†	4	3	0.10	0.08–0.12	3	0.12	0.11–0.13
	2	3	0.09	0.07–0.10			
Brome							
1951	8	4	0.21	0.18–0.22	4	0.23	0.20–0.24
1952†	8	3	0.13	0.10–0.15	3	0.09	0.07–0.12
	4	3	0.14	0.10–0.16			
Oats							
1952†	8	6	3.39	2.71–4.44	6	3.90	3.13–4.98
	4	6	3.56	2.42–4.76			
Red Clover							
1952	4	4	0.15	0.13–0.18	4	0.13	0.11–0.16
Sweet Clover							
1951	4	3	0.09	0.08–0.10	2	0.10	0.08–0.11
1952	4	6	0.16	0.12–0.29	6	0.13	0.12–0.19
Timothy							
1952†	4	3	0.14	0.11–0.19	3	0.11	0.09–0.12
	2	3	0.11	0.11–0.12			
White Dutch Clover							
1951	4	2	0.08	0.07–0.09	2	0.09	0.08–0.09
1952	4	10	0.10	0.08–0.14	10	0.11	0.07–0.14

Weeds							
Canada Thistle							
1951	8	4	0.13	0.12–0.15	4	0.13	0.08–0.16
1952	8	9	2.64*	0.56–5.01	9	1.36*	0.41–2.90
Dandelion							
1951	4	2	3.11	2.67–3.55	2	1.73	1.69–1.76
1952	8	9	1.15	0.76–1.48	9	1.12	0.62–1.54
Lamb's Quarters							
1951	8	4	3.83	1.49–6.23	4	3.08	2.22–4.51
1952†	8	6	7.88	5.92–8.83	6	9.56	8.45–10.40
	4	6	8.15	7.15–8.77			
Redroot Pigweed, 1951							
From hog run	8	2	4.62	4.47–5.77	2	5.63	5.12–6.14
From field	8	2	1.61	1.46–1.75	2	0.79	0.71–0.86
Russian Pigweed							
1952	8	9	4.38**	2.49–5.56	9	2.49**	1.39–4.76
Russian Thistle							
1951	8	2	4.67	4.49–4.85	2	4.28	3.78–4.78
Stinkweed							
1952	8	6	1.16	0.42–3.00	10	0.96	0.25–1.81
	4	6	1.39	0.20–3.32			
	2	8	1.57	0.24–2.80			
Wild Mustard							
1952	4	9	0.32	0.14–0.76	9	0.29	0.13–0.76

† Plots were laid out in Latin squares.
* Significant beyond the 5 per cent point.
** Significant beyond the 1 per cent point.

TABLE 2.—ANALYSIS OF VARIANCE IN RUSSIAN PIGWEED DATA

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	17	35.35	—	—
Treatments	1	16.15	16.15	10.11**
Dates	2	0.73	0.37	< 1
Interaction (Treatments × Dates)	2	1.76	0.88	< 1
Replicates	2	0.73	0.37	< 1
Error	10	15.98	1.598	—

**Significant beyond the 1 per cent point.

equivalent per acre the treatment resulted in only a slight stunting of th dandelions in these plots. The nitrate content of wild mustard was low in samples from treated and untreated plots.

ACKNOWLEDGMENT

The authors are indebted to members of the Soils Department, University of Alberta, for providing the spectrophotometer used and for assistance in developing the method of analysis.

REFERENCES

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. Washington, D.C. 6th ed. 1945.
2. Bradley, W. B., H. F. Eppson, and O. A. Beath. Livestock poisoning by oat hay and other plants containing nitrate. Wyoming Exp. Sta. Bull. 241. 1940.
3. Eckerson, Sophia. Protein synthesis by plants. Bot. Gaz. 177 : 377-390. 1924.
4. Mayo, N. S. Cattle poisoning by nitrate of potash. Kansas Agr. Exp. Sta. Bull. 49. 1895.
5. Olsen, O. E., and E. Whitehead. Nitrate content of some South Dakota plants. Proc. South Dakota Acad. Sci. 20 : 95-101. 1940.
6. Snedecor, G. W. Statistical methods. Iowa State College Press. 4th ed. 1946.
7. Snell, F. D., and Cornelia T. Snell. Colorimetric methods of analysis. D. Van Nostrand Co. Inc., New York. 1936.
8. Stahler, L. M., and E. I. Whitehead. The effect of 2,4-D on potassium nitrate levels in leaves of sugarbeets. Sci. 112 : 749-751. 1950.
9. Thorpe, F., Jr. Further observations on oat hay poisoning. J.A.V.M.A. 92 : 159-170. 1938.

INFLUENCE OF SOIL ZONE ON THE CHEMICAL COMPOSITION OF CEREALS IN ALBERTA

J. D. Newton[1]

University of Alberta, Edmonton, Alberta

[Received for publication November 6, 1952]

ABSTRACT

The average protein content of grain grown in Alberta *is not* directly related to the nitrogen content of the soil, as between the brown and black soil zones, but *is* directly related as between the black and grey soils. The grain protein decreases from the drier to the moister zones. This has an important bearing on feeding value of grain and malting quality of barley grown in different zones. No direct relationship has been found between total phosphorus content of soil and crop. The black soils are generally highest in total phosphorus and the grey lowest. The total sulphur content of grains grown on sulphur-deficient grey wooded soils is relatively low, but no consistent difference is found between the grains grown on brown and black soils. There is no consistent relationship between total calcium or magnesium contents of soil and grain. The magnesium content of the grain is much higher than the calcium, and the oats are considerably higher in calcium than the wheat or barley. The potassium content of the wheat, barley, and oats grain is quite variable and relatively low in all zones by comparison with general averages.

A project was undertaken at the University of Alberta with the object of studying the composition and nutritive value of foods and feeds (mainly cereal grains) grown on representative soils of the main agricultural soil zones of Alberta. Fertilizers were not applied to any of the crops studied. The Departments of Soils, Plant Science, Animal Science, and Chemistry co-operated in the work. This made it possible to approach the investigation from many angles. *First*, the soil of the fields on which some of the crops were grown was analysed. *Second*, the grain grown on these soils was analysed for various constituents. *Third*, the feeding value of some of the grain crops was studied in livestock feeding experiments.

SOILS AND CROPS

The soils and crops included in this study were analysed separately for various elements, and the column heights in Figure 1 represent the average values for each soil zone. All results are expressed on a water-free basis.

Standard or widely recognized methods of chemical analysis for total constituents were used, but special mention should perhaps be made of sulphur and potassium. For the determination of sulphur in the soil samples sodium carbonate fusion was used, and calcium carbonate fusion for potassium (7). Soil and plant sulphur was precipitated as barium sulphate, and potassium as platinic chloride. These methods had been tested and used extensively in earlier work in this laboratory.

In the brown soil zone the following four soils were analysed: Legend and Conrad loams, Wrentham and Taber silt loams. In the black soil zone the following nine were analysed: Mundare, Edmonton, Spruce Grove, Legal and Bon Accord loams, Morinville clay loam, Bremner, Clover Bar

[1] Professor of Soils.

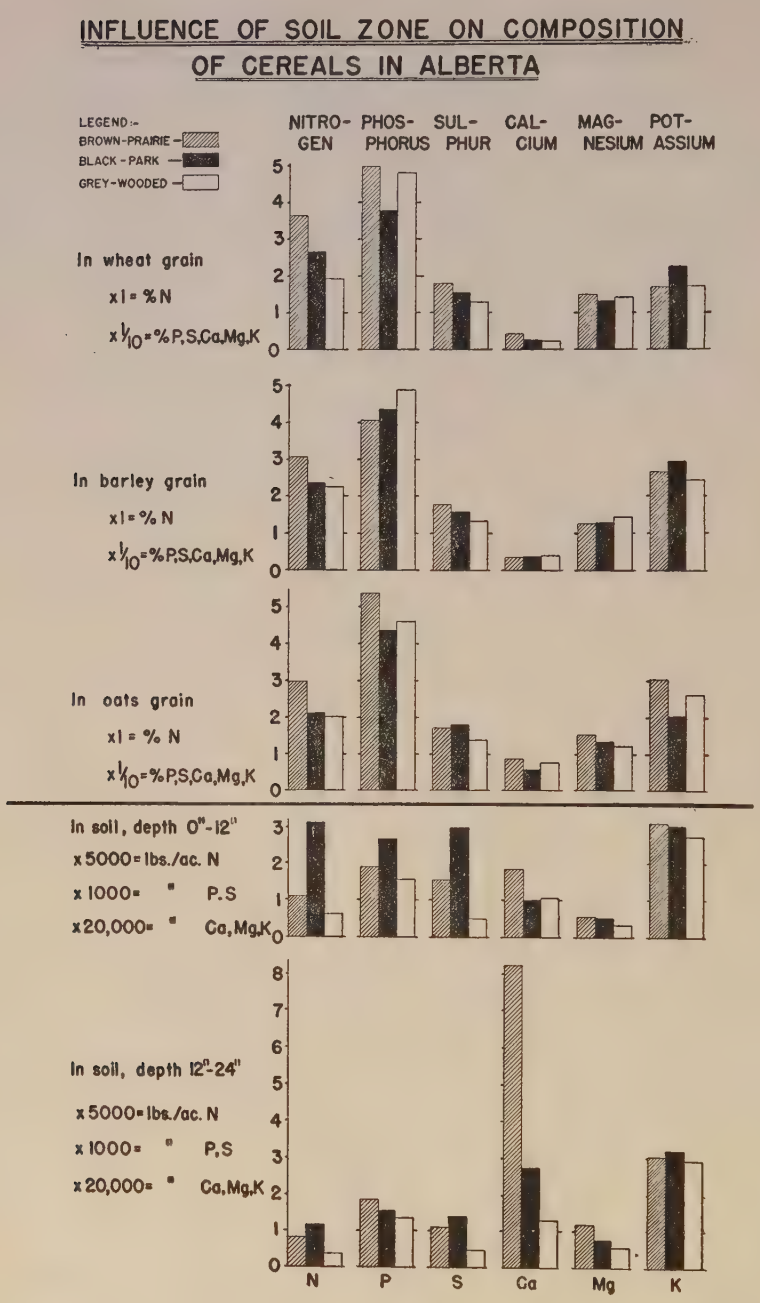

INFLUENCE OF SOIL ZONE ON COMPOSITION OF CEREALS IN ALBERTA
LEGEND:-
BROWN-PRAIRIE
BLACK-PARK
GREY-WOODED
NITRO-GEN
PHOS-PHORUS
SUL-PHUR
CAL-CIUM
MAG-NESIUM
POT-ASSIUM
In wheat grain
x1 = % N
x 1/10 = % P,S,Ca,Mg,K
In barley grain
x1 = % N
x 1/10 = % P,S,Ca,Mg,K
In oats grain
x1 = % N
x 1/10 = % P,S,Ca,Mg,K
In soil, depth 0"-12"
x 5000 = lbs./ac. N
x 1000 = " P,S
x 20,000 = " Ca,Mg,K
In soil, depth 12"-24"
x 5000 = lbs./ac. N
x 1000 = " P,S
x 20,000 = " Ca,Mg,K
N
P
S
Ca
Mg
K

FIGURE 1.

TABLE 1.—GENERAL AVERAGE COMPOSITION OF CEREAL GRAINS*

	Nitrogen N%	Phosphorus P%	Sulphur S%	Calcium Ca%	Magnesium Mg%	Potassium K%
Wheat	2.11	0.39	0.20	0.04	0.14	0.42
Barley	2.03	0.37	0.15	0.06	0.13	0.49
Oats	1.92	0.34	0.21	0.09	0.14	0.43

* Morrison, F. B. Feeds and feeding. 21st ed. 1949.

and St. Albert clays. In the grey wooded soil zone the following two were analysed: Warburg loam and Fallis silt loam. It should be noted that these names represent Alberta stations close to the fields sampled, and not soil series names.

The grain crops grown on these fields were analysed and the crops were used in feeding experiments by McElroy *et al.* (3). The grain analysis columns of Figure 1 represent averages for the 1944, 1945 and 1946 crops from the black soil field locations given above, and for the 1944 and 1945 crops from the brown and grey wooded soil fields. These were representative crop years in Alberta, neither extremely good nor extremely poor, and not very far from long-time averages. Varieties of grain grown were Marquis and Thatcher wheat, Banner and Victory oats, Newal and Olli barley. Many analyses (2) had shown, however, that the climate-soil zone is the important factor determining average nitrogen content, rather than crop variety.

The grain composition averages given in Table 1 were obtained from Morrison's widely known compilation entitled "*Feeds and Feeding*", and represent the averages of grains grown over a wide area. The averages for the different Alberta soil zones shown in Figure 1 may, of course, be compared with any special data as well as with Morrison's averages. Comparable Canadian data for grain composition are apparently largely confined to nitrogen.

NITROGEN

Figure 1 shows that the wheat, barley and oats of the brown soil zone are all very high in nitrogen or protein, and that the wheat of the grey wooded soil zone is slightly low in nitrogen by comparison with the general averages shown in Table 1. The barley and oats are not low in protein in these experiments even when grown on grey wooded soils, but much other analytical data obtained by the University of Alberta Departments of Soils and Plant Science show that the protein content of barley, like that of wheat, tends to decrease from the drier to the moister zones. A great many determinations made by the Board of Grain Commissioners for Canada (1) have shown this trend. The relatively low protein content of wheat grown on grey soils is also shown by long-time averages published by the Department of Soils (5).

The average nitrogen or protein content of the wheat, barley and oats grown in Alberta, as shown in Figure 1, *is not* directly related to the nitrogen content of the soil as between the brown and black soil zones but *is* directly related as between the black and grey wooded.

The milling industry is vitally interested in protein content and other variations in the composition of wheat, because of their effects on milling and baking quality, and the brewing industry also is greatly concerned with protein content and other variations in the composition of barley, because of their influence on malting quality. But, as pointed out by McElroy *et al.* (3) relatively little study has been made of the question of the effect that differences in protein content of grains may have on their feeding value for livestock.

The results of pig-feeding experiments with grain grown in the different soil zones of Alberta, reported by McElroy and Draper (4), show that, when high protein grains are used in the ration, good results can be obtained by adding substantially smaller quantities of protein supplement than are required when the grains used are of low protein content. Thus more supplement would normally be required in the grey soil zone than in the black, and less in the brown.

The brewing industry, on the other hand, prefers a barley that is low in nitrogen because high malting quality is usually associated with low protein. Barley grown in Alberta's grey wooded soil zone and in the moister parts of the black soil zone is therefore generally accepted for malting rather than barley from the brown and thin black soil zones.

PHOSPHORUS

In two of the three grains, the phosphorus content is lower in the black soil zone than in the brown and grey. The exception in the case of barley limits definite conclusions with respect to phosphorus content on the basis of these data. Nevertheless, other data obtained by the Department of Soils also indicate that grain grown on Alberta's black soils tends to be lower in phosphorus than grain from the other zones.

There is no direct relationship between the total phosphorus content of the soil and that of the crop (see Figure 1). The black soils, which are highest in total phosphorus, especially in the surface foot, do not produce grain of exceptionally high phosphorus content.

SULPHUR

There is evidently some correlation between the sulphur content of the soil and that of the grain. The total sulphur content of the grey wooded soils is low and likewise that of the grains grown on these soils as shown in Figure 1. Similar relationships were found by T. W. Peters (6). The differences between the grains grown in the brown and black soil zones are not as definite. The black soils themselves tend to be highest in sulphur, probably because of their high organic matter content, but in some cases the subsoil in the brown zone is very high in sulphur because it contains much gypsum.

The sulphur content of wheat, barley and oats grown on grey wooded soils is quite low by comparison with general averages, whereas that of the black and brown soil grain is closer to average.

CALCIUM AND MAGNESIUM

The total calcium content of the brown soils is much higher than that of the black and grey wooded soils, and the magnesium also is somewhat higher in the brown, but there is no consistent evidence in these experiments of a direct relationship between the total calcium or magnesium content of the soil and that of the grain. The oats are considerably higher in calcium than the wheat and barley, probably because of their heavier hulls. Barley is generally a better source of calcium than wheat, and oats a much better source.

The wheat, barley and oats grown on grey wooded and black soils in these experiments are all relatively low in calcium by comparison with the general averages shown in Table 1. On brown soils the disparity was not so great except in the case of barley. The magnesium content of the grain is much higher than that of the calcium, and about average with respect to this constituent. There is no consistent difference in the magnesium content of the wheat, barley and oats.

POTASSIUM

The potassium content of the wheat, barley and oats grain is somewhat variable. In all cases the soils are high in total potassium and apparently there is no consistent relationship between that of the soil and grain. The potassium content of barley and oat grain is generally higher than that of wheat, and this is probably because their hulls are relatively rich in this element. By comparison with the general averages given in Table 1 the potassium content of wheat, barley and oats grown in Alberta is quite low.

ACKNOWLEDGMENTS

This investigation was conducted with the aid of a grant from the Committee on Agricultural Research Grants, University of Alberta.

The University Department of Soils was responsible for the soil and grain analyses reported in this paper with the exception of the nitrogen determinations on the grain samples, which were made by the Department of Plant Science. Doris L. Williams and E. R. Edwards conducted most of the analyses used in the preparation of this paper.

REFERENCES

1. Board of Grain Commissioners for Canada. Annual reports of the grain research laboratory, Winnipeg, Man. 1939-48.
2. McCalla, A. G., and Dyson Rose. The quality of Alberta-grown wheat. Univ. of Alberta Bull. 37. 1941.
3. McElroy, L. W., W. Lobay, and R. D. Sinclair. Feeding grains of different protein content. J. An. Sci. 7 : 494-500. 1948.
4. McElroy, L. W., and H. H. Draper. Feeding grains of different protein content to growing pigs. Sci. Agr. 29 : 579-583. 1949.
5. Newton, J. D., A. S. Ward, and C. F. Bentley. Wooded soils and their management. University of Alberta, Faculty of Agriculture, Bull. 21, 4th ed. 1948.
6. Peters, T. W. The effect of fertilizers on the sulphur content of soil and crop plants. M.Sc. thesis, University of Alberta. 1942.
7. Robinson, W. O. Method and procedure of soil analysis used in the division of soil chemistry and physics. U.S.D.A. Circ. 139. 1939.

THE RELATION OF LIGNIN, CELLULOSE, PROTEIN, STARCH AND ETHER EXTRACT TO THE "CURING" OF RANGE GRASSES[1]

W. J. PIGDEN[2]

Experimental Station, Swift Current, Sask.

[Received for publication November 12, 1952]

ABSTRACT

Two grasses native to Western Canada, namely, common speargrass *Stipa comata* Trin. and Rupr. and rough fescue *Festuca scabrella* Torr., which "cure" well, i.e., withstand weathering and retain a relatively high nutritive value after drying in the fall and hence provide satisfactory winter grazing, are compared at four stages of maturity with two common introduced species, namely, smooth bromegrass *Bromus inermis* Leyss. and crested wheatgrass *Agropyron desertorum* (Fisch.) Shult., which lack wholly or in part this useful characteristic.

Histological studies of cross sections indicate: (1) at maturity lignification of the introduced species involved not only the vascular bundles but the surrounding tissues as well, whereas lignified areas in the native species were confined chiefly to the vascular bundles; (2) large numbers of cellulose fibres enable the native grass leaf to withstand long periods of weathering; (3) at maturity the native grass leaves contain numerous fat globules in the mesophyll cells.

Chemical analyses for these three fractions confirm the histological observations. No association has been detected between protein or starch content and ability to cure.

Lignification is considered to be the most important single factor contributing to the curing property. The position and extent of the lignified tissues appear to be more important than the quantity present.

INTRODUCTION

The major portion of the forage requirements for the extensive livestock industry in the provinces of Manitoba, Saskatchewan and Alberta is supplied by native pasture lands which have been estimated to occupy at least 40 million acres (3).

A considerable proportion of this acreage is utilized for its standing forage during the fall and winter months. This is possible for two main reasons:

1. The warm chinook winds which usually partially clear the snow from the pastures and moderate the temperature in many areas.
2. Several grasses common to the prairies of Western Canada retain a relatively high nutritive value during late summer and throughout the subsequent fall and early winter. In addition, they preserve their physical form; stems and particularly leaves do not decompose to any extent for eight to ten months after growth ceases. Ranchers refer to this remarkable property as "curing".

   Curing normally occurs during late July but may also take place in mid-June or late August, depending on the season.

Ranchers report that cured forage maintains sheep and cattle in a thrifty condition during the winter with little or no supplemental feeding, despite the fact that protein content of the cured forage is usually less than

[1] Contribution from Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada. Part of an M.Sc. thesis submitted to the Department of Plant Science, University of Alberta, Edmonton, Alta.

[2] Technical Officer, Forage Plants Section, Experimental Station, Swift Current, Sask.

5 per cent (3). From the viewpoint of the rancher, curing is an invaluable asset because it decreases production costs by shortening materially the winter feeding period.

As stated previously, the bulk of the forage for livestock production on the prairie is derived from native grass pastures. An increasing acreage of unproductive land, including depleted pastures, submarginal cultivated lands and abandoned farms, is however being regrassed with comparatively high-yielding introduced grasses (2). Several of these grasses show little or no tendency to cure, a feature which limits their usefulness in areas where winter pasture is required. A grass is required which incorporates the curing property with high yield and other desirable characteristics. It is the purpose of this study to elucidate some of the factors involved in the curing process.

The following native grass species are credited with the ability to cure to a greater or less degree:

Common Name	*Scientific Name*
1. Common speargrass	*Stipa comata* Trin. and Rupr.
2. Blue grama grass	*Bouteloua gracilis* (H.B.K.) Lag.
3. Western wheatgrass	*Agropyron Smithii* Rydb.
4. Rough fescue	*Festuca scabrella* Torr.
5. Parry's oatgrass	*Danthonia Parryi* Scribn.
6. Short awned porcupine grass	*Stipa spartea var. curtiseta* Hitchc.
7. Niggerwool	*Carex filifolia* Nutt.
8. June Grass	*Koeleria cristata* Pers.
9. Northern wheatgrass	*Agropyron dasystachyum* (Hook.) Scribn.

In addition several grass species of minor importance, as well as certain forbs, particularly salt sage *Atriplex Nuttallii* S. Wats., and winterfat *Eurotia lanata* (Pursh) Moq., may have this property.

These species are dominant in the prairie area within the provinces of Manitoba, Saskatchewan and Alberta. The region of distribution extends to the Foothills on the west and to the southern boundary of the Park Belt in the north and east.

LITERATURE REVIEW

Clark and Tisdale (3) have reported the chemical analysis of about 1000 samples of native vegetation from the area concerned. Data on the chemical composition of the most important cultivated species within this area are also available (3, 19). This information does not reveal differences to account for the superior nutritive value of cured native grass forage as compared with cultivated grasses. However, the data are based on the conventional proximate principles analysis (28), and this system suffers from serious shortcomings. In this connection much of the lignin, a relatively indigestible constituent of feeds, is included with the nitrogen-free extract, the supposedly readily digestible fraction (30, 31). Conversely, crude fibre which is normally considered to be the woody, poorly digested portion, may have a coefficient of digestibility fully equal to that of the nitrogen-free extract (6, 31).

Substitution of the crude fibre and nitrogen-free extract proximate analysis by determinations for lignin, cellulose and other carbohydrates has been advocated by Crampton and Maynard (6). When compared with the

Weende method most of the results from the proposed modification agreed much more closely with actual digestibility studies (4, 5, 6, 13, 17, 22, 26); hence it seems likely that it would provide a more accurate index of the actual nutritional value of a given feed.

Because forages contain relatively large amounts of lignin it is considered to be an important factor in their utilization. Numerous studies show that in general the digestibility of a feedstuff is associated with its lignin content, and usually a high negative correlation exsists between percentage lignin and the dry matter digestibility coefficient (5, 9, 12, 15, 20, 36).

Other investigations have established the fact that normally the lignin content of forages increases steadily with advancing maturity (4, 5, 9, 32, 34, 35). There is also evidence that environmental factors may affect the deposition of lignin in the plant; for example, unusually dry or cold weather seems to cause abnormal increases in lignin content at earlier growth stages (27, 35).

Wide variations in lignin content may exist between closely related species. According to Patton and Gieseker (35) mountain bromegrass *Bromus marginatus* Nees. and crested wheatgrass Fairway type, *Agropyron cristatum* (L.) Gaertn., have a much lower lignin content than smooth bromegrass *Bromus inermis* Leyss., and crested wheatgrass, Standard type *Agropyron desertorum* (Fisch.) Schult., respectively.

There is reason to believe that factors other than the mere quantity of lignin present may be involved. Crampton and Forshaw (5) noted a marked decrease in the digestibility of pasture herbage over the short period of ten days with only small increases in lignin content. Drapala *et al.* (9) followed the process of lignification in red clover by histological methods. They observed that lignification increases steadily with advancing maturity. Regions around vascular bundles were mainly involved and xylem parenchyma underwent lignification at maturity, thus making the food reserves in these cells partially or wholly unavailable to the animal.

These important observations indicate that most of the lignin, in some forages at least and up to a certain stage of maturity, is deposited in the vascular bundles. At this point lignin begins to infiltrate and encrust the walls of adjacent tissues and may act as a physical barrier to prevent the utilization of cellulose. This in turn may hinder enzymatic digestion of more soluble nutrients within the cell (27, 32).

In this connection, cellulose in a relatively pure form is claimed to be highly digestible by ruminants (22, 23, 25). On the other hand a cellulose associated with lignin and other substances in forage is believed to be digested to a much smaller extent (14, 22, 23, 33, 35). When red clover at various stages of maturity was fed to sheep and the feces residues examined microscopically, it was found that size of particle of the undigested material was directly proportional to the degree of lignification and inversely proportional to the digestibility coefficient (9).

In addition to the effect of increases in the more indigestible constituents the nutritive value of cured forages may be affected by leaching. Guilbert and Mead (16), showed that large losses of soluble nutrients occurred from bur clover when the stem-cured forage was exposed to heavy

rains. Norman (32) reported that rye grass, *Lolium italicum* var. Western Wolths, contained as much as 34 per cent water-soluble fructosan at an early growth stage. The early work of Le Clerc and Breazeale (21) suggests that largest losses of soluble nutrients by leaching occur when the plants have reached maturity.

Translocation of soluble reserves from the aerial parts of the plant to the roots late in the season may also be a factor. Rapp (37) found that Johnson grass stored most of its carbohydrate reserves in the stem as sucrose during the growing season and transferred it to the roots at maturity.

Since the protein content of cured western native grasses is very low (3), this fraction obviously contributes little towards the energy requirements of the range animal. There is some evidence to suggest, however, that the protein requirement for efficient roughage digestion is quite low, unless starch or starchy grains are included in the ration (1). According to Louw *et al.* (23), old dry winter pasture in South Africa with about 3 per cent protein is apparently not deficient in nutrients required by ruminal organisms responsible for the breakdown of cellulose, to a limit presumably set by the degree of lignification.

The digestibility and energy value of the ether extract fraction of forages is usually fairly low in comparison to the ether extract of seeds, because of the inclusion of many fat soluble, non-lipid substances. Hence, this fraction is normally considered to be of minor importance.

MATERIALS AND METHODS

Grasses

Since this investigation was regarded as being of a preliminary nature, the grasses were selected to represent a fairly wide range of grass types found under various growing conditions. In this manner it was hoped to gain general information which would be of value for planning future investigations. The following grasses, accompanied by a brief description of each, were chosen for comparative studies:

Native Grasses

(1) Common speargrass *Stipa comata* Trin. and Rupr. This is an extremely xerophytic grass, the second dominant in the short-grass prairie and a dominant species in the mixed prairie association. Because of its relative abundance, palatability and marked ability to cure, it is one of the most important grass species for winter forage in the prairie region.

(2) Rough fescue *Festuca scabrella* Torr. A less xerophytic grass than speargrass, favouring a cooler climate with better moisture conditions. It is the dominant species in the submontane prairie association, producing an abundance of excellent cured forage in the Cypress Hills and Rocky Mountain Foothills.

Cultivated Grasses

(1) Crested wheatgrass (Standard type), *Agropyron desertorum* (Fisch.) Schult. This grass has been extensively used for regrassing programs in the dryland areas of the Prairie Provinces because of its drought resistance, winter hardiness, and ability to compete with weeds. It is considered inferior to common speargrass or rough fescue as a stem-cured forage although it resists weathering fairly well.

(2) Common or smooth bromegrass *Bromus inermis* Leyss. This grass is not as winter-hardy or drought-resistant as crested wheatgrass, but it is used considerably for regrassing where moisture conditions are more favourable. As a stem-curved forage, it is regarded as being distinctly inferior to the three grasses previously discussed and appears to be of little nutritional value to the range animal when grazed during the winter months.

In addition to the four main species listed above, two grasses of minor importance found on the common speargrass site, namely, sandgrass *Calamovilfa longifolia* (Hook.) Scribn., and Indian ricegrass *Oryzopsis hymenoides* (Roem. and Schult.) Ricker, were included in the histological study but only to a limited extent in the chemical analysis.

Hereafter, the grasses will be referred to in the text as speargrass, fescue, crested wheatgrass, bromegrass, sandgrass and ricegrass.

Sampling

A typical sampling site for each of the four main species was selected within the mixed prairie area in the Swift Current district. Each site occupied about three-quarters of an acre.

To assure that a representative sample was obtained from each site, two base lines were established at right angles to each other along two sides of each experimental area. The site was then mapped out on paper and divided into numbered square meter units. Twelve of these metre quadrats were selected at random. Each quadrat was established within the area by measuring inward from the two base lines; the corners were marked with spikes. The quadrats were again subdivided into quarters, a different quarter being cut at each stage of growth. Not all the quarter quadrats contained the species being sampled but never less than eight were cut at any stage of growth.

In order to study changes which occur as the plant develops and matures the grasses were sampled at several stages of growth. Sampling of different species at comparable growth stages was considered preferable to sampling on set dates. The five stages employed by Patton (34) for lignin and cellulose studies in Montana were chosen. They are as follows:

1. Early leaf growth.
2. Flowering culms in full bloom.
3. Seeds ripe.
4. Cured (most of the seeds fallen, plants dried on the stem).
5. Remnants of the previous year's growth (sampled in early spring).

The fifth growth stage was considered to be relatively unimportant; hence, only a few analyses and tests were carried out.

Hereafter, growth stages will be referred to by number, or as cured (Stage 4).

The grasses were sampled in the afternoon because of diurnal variations known to exist in the chemical composition of plants, usually between 2.00 p.m. and 5.00 p.m. They were cut approximately one inch above the crown, except for fescue which was cut at the top of the rough basal growth. The old growth was separated from the current year's growth and discarded. Samples were divided into leaf and stem, leaving the leaf sheath on the stem. Stem leaves of speargrass were included with basal leaves. To make sure that the ripe seeds would not interfere with the qualitative starch test, the grass heads were removed at Stages 3 and 4 and analysed separately. Fescue stems were not analysed at any stage because very few culms were found within the sampling site.

The quantity of forage dried for chemical analysis was limited to approximately 500 grams (fresh weight) by the size of the drying units. Accordingly, the clippings from the quarter quadrats at each growth stage were bulked, well mixed, and a composite sub-sample taken for drying.

A typical leaf and stem were selected for sectioning from each quadrat on every sampling site at all growth stages.

Since it was planned to use these samples for soluble carbohydrate tests as well as lignin determinations, a method of drying suitable for both purposes was required. Heat drying, at a temperature which would inactivate enzymes, may result in a marked increase in apparent lignin content (24). Air drying, while satisfactory for lignin determinations, permits cell respiratory activities to continue with a consequent loss of readily available carbohydrate (8). Hence, both of these methods were considered to be unsatisfactory.

The lyophilization (freeze drying) technique of Davies *et al.* (7) is claimed to be capable of removing moisture from small and medium sized forage samples with minimal changes occurring in their chemical composition. It was selected as the most suitable drying method available with the apparatus modified as follows: The Kilner jar and Thermos flask described by Davies *et al.* (7) for field collecting and freezing samples was replaced by a one-gallon, insulated ice-cream pack (available from dairy supply houses), and a metal can, 13 inches high by 4 inches in diameter, which served as the inner container, with ample room for a charge of powdered solid CO_2 around it. The Thermos flask, used as a container for the dry ice-acetone mixture which chilled the condenser while drying the sample, was replaced by an ordinary one-half gallon metal can, insulated on the outside with a layer of shredded paper two to three inches thick. Although not so economical in the use of dry ice as Thermos flasks, the two substitutes served the purpose very well.

Difficulties encountered while using the lyophylization technique were mainly concerned with insufficient vacuum. A vacuum pump in good condition, rated by the manufacturer as capable of developing vacuum of 0.0004 mm. mercury, was found to be satisfactory.

It was also found that thick bromegrass stems took at least twice as long to dry as did leafy material. A method of shredding such material before freezing would speed up drying considerably.

Experience gained while using the freeze dry method indicates that it is quite suitable for drying a small number of samples for specialized research purposes. In its present form it is too time- and labour-consuming for routine purposes, where large numbers of samples are involved.

The samples were ground in a small laboratory hammermill so that 95 per cent or more would pass through a 60-mesh screen. They were stored in air-tight containers (half-pint sealers with rubber rings) at a moisture content approaching that of air-dried material.

Biochemical Determinations

Lignin

The 72 per cent sulphuric acid method of Ellis *et al.* (13) was used as published. Recent studies completed by Ellis (12) indicate that deter-

minations made using this method show a reasonably satisfactory degree of reproducibility both within and between laboratories.

Cellulose

At present there is no general agreement on a satisfactory method for nutritional studies. The method of Druce and Willcox (10), which is claimed to isolate primarily hexosen cellulose, was selected as being the most suitable.

The method was modified slightly. Filter sticks were substituted for the poplin-Buchner funnel method of filtration, and Gooch crucibles with asbestos mats were used for ashing instead of the silica crucibles specified by the authors. Use of the filter sticks permitted all filtering and washing operations to be carried out in one beaker, thus eliminating transfers.

Ether Extract (Crude Fat)

Crude fat analyses were carried out by the official A.O.A.C. (28), procedure. Since most feed laboratories now substitute petroleum ether for ethyl ether as the extracting solvent, the method was modified to this extent.

Crude Protein

The standard A.O.A.C. (28) Kjeldahl method for determining crude protein (total nitrogen $\times$ 6.25) was used. One-quarter part of selenium was added to the regular salt mixture. The boric acid modification of Scales and Harrison (39) was used in the distillation procedure.

Starch

The qualitative method used by Ekelund (11) to test Swedish hays for starch was used.

Histological Techniques

Sectioning

A free-hand method was employed so that microchemical stains could be applied to fresh tissue. Leaves, both basal and stem, were sectioned at a point approximately half-way between leaf tip and leaf base. In the case of stem leaves the second leaf from the top was used. Stems, including the leaf sheaths, were sectioned at a point about half-way between the two uppermost nodes. As the sections were cut they were dropped into 70 per cent ethyl alcohol and left from 30 to 60 minutes to dissolve out the chlorophyll. This last step was found to be essential to obtain a sharp, clear differentiation of tissues when the lignin test was applied.

Staining

After the chlorophyll had been removed, the sections were transferred to a slide and stained. The hydrochloric acid, phloroglucin method of Johannson (18), was applied to cross sections of leaf and stem at all stages of maturity to differentiate the lignified tissues. The Sudan IV lipid test outlined by Johannson was used on cross-sections of the native leaves at Stages 4 and 5.

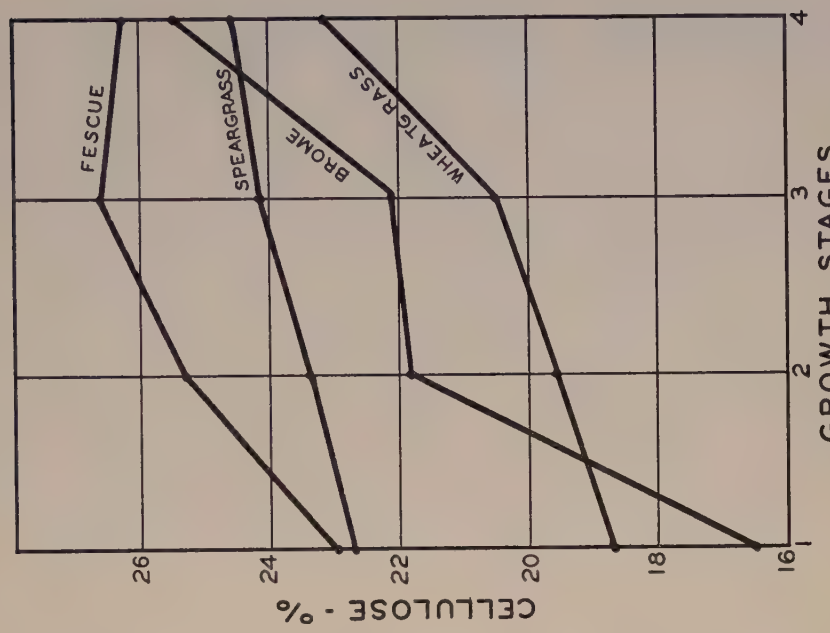


FIGURE 2. Effect of growth stage on cellulose content.

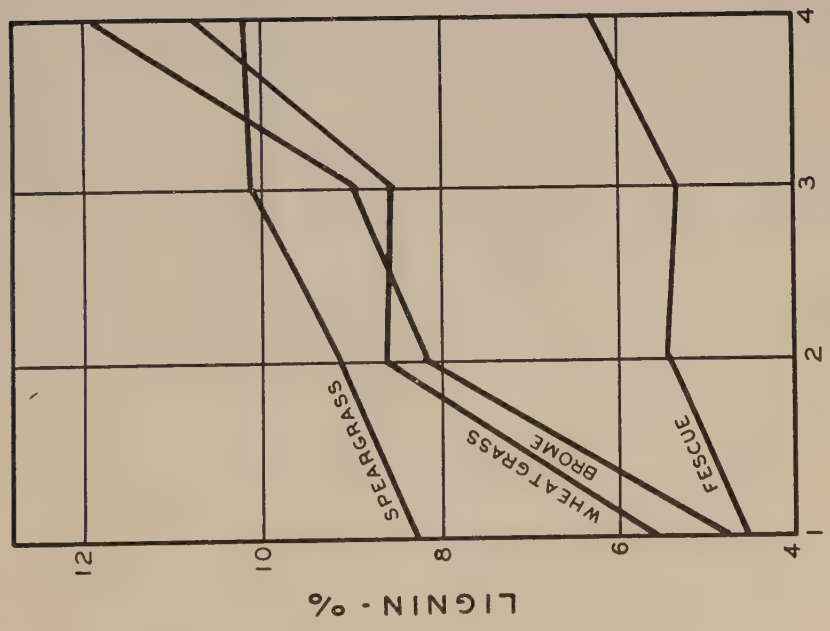


FIGURE 1. Effect of growth stage on lignin content.

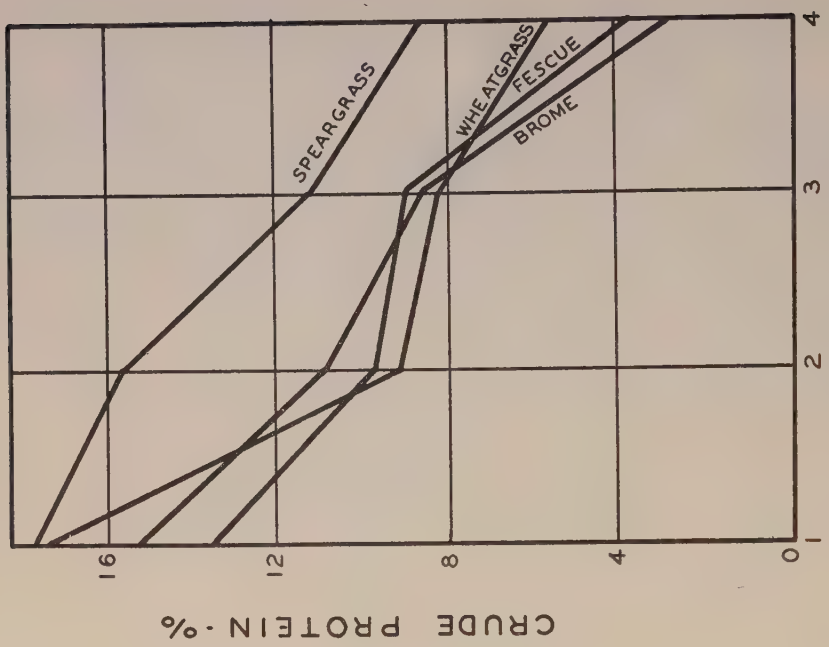


FIGURE 4. Effect of growth stage on protein content.

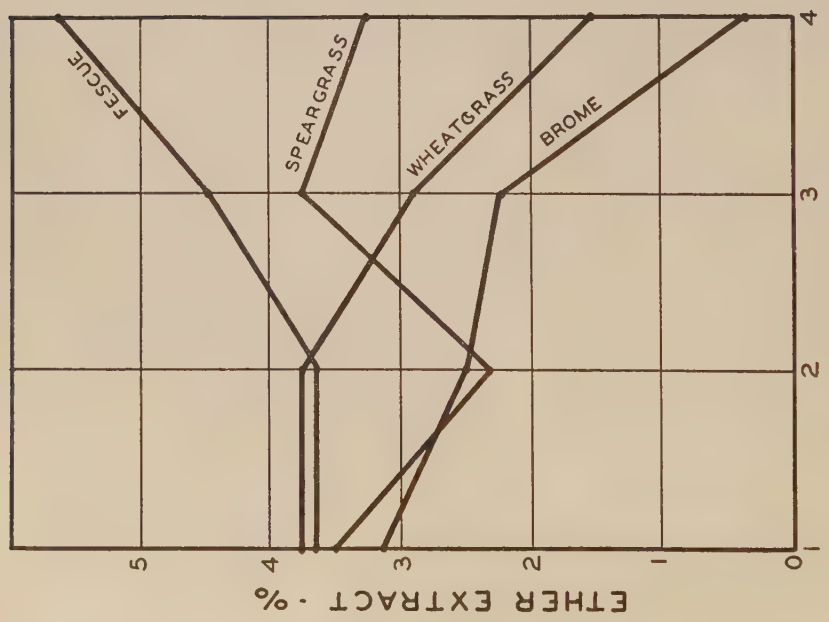


FIGURE 3. Effect of growth stage on ether extract content.

Photomicrography

The stained sections were examined under low power, using a small microscope. Several of the most uniform and representative of the group were quickly changed to a clean slide, mounted in a glycerol water solution and photographed with small eyepiece camera on a separate microscope. Since the phloroglucin stain fades quite rapidly, the last two steps must be carried out promptly.

Very few of the sections tested for fat were photographed as in most cases the unstained fat globules showed up equally well or better in those sections stained for lignin and, in addition, more contrast was obtained in the photomicrograph.

RESULTS

On the basis of ratio of leaf to stem and anatomical structure, the grasses fall into two fairly distinct groups. Table 1 shows that 95 per cent or more of the dry weight of the native grasses at Stage 4 consisted of leaves. Conversely, approximately 90 per cent of the dry weights of the cultivated grasses at Stage 4 consisted of stems. Therefore, the most important comparisons are those between material from native grass leaves on the one hand and that from cultivated grass stems on the other.

TABLE 1.—RELATIVE PROPORTIONS OF LEAF TO STEM IN PERCENTAGE OF DRY WEIGHT

Growth stage	Grass	Leaf	Stem	Head
		%	%	%
2	Speargrass	91.0	9.0	—
	Fescue*	98.0	2.0	—
	Crested wheatgrass	15.0	85.0	—
	Brome	24.0	76.0	—
3	Speargrass	93.0	7.0	—
	Fescue*	98.0	2.0	—
	Crested wheatgrass	16.0	66.0	18.0
	Brome	15.0	65.0	20.0
4	Speargrass	96.0	4.0	—
	Fescue*	98.0	2.0	—
	Crested wheatgrass	13.0	87.0	—
	Brome	9.0	91.0	—

* Fescue not weighed, estimated only.

Although separate chemical analyses were made on leaf and stem, the results were recalculated to a whole plant basis and with the exception of Stage 5 are reported graphically in Figures 1, 2, 3, and 4.

Lignin

Figures 5 and 7 show that the lignified tissue in the immature native grass leaf was confined chiefly, if not entirely, to the vascular bundles. Furthermore, in the fescue leaf (Figure 5), these bundles were smaller in size and less numerous than they were in the speargrass leaf, thus constituting a smaller proportion of the total cross-sectional leaf area. In addition,

Figures 6 and 8 indicate that, as the plant matured, lignin apparently did not infiltrate and encrust the adjacent cellulose fibres to any great extent. Neither was there any sign of lignification in the walls of the mesophyll cells. Chemical analyses corroborated these observations, the initial lignin content of fescue was much lower than that of speargrass (Figure 1), and the slope of both curves was similar and relatively flat which indicated that no large increase in lignin content occurred during the season.

The two cultivated grasses consisted entirely of leaf and stem bases at Stage 1 and were relatively low in lignin content. As the plants reached a more mature stage, a reversal of the ratio of leaf to stem occurred and the preponderance of stem to leaf became more marked as the season advanced (Table 1). In the early stages of stem growth, Stage 2 (Figures 9 and 11), the lignification of the cell walls was mostly confined to the vascular bundles. At a later growth stage, however, Stage 4 (Figures 10 and 12), heavy lignification occurred in the walls of the fibres and the parenchyma adjacent to the vascular bundles. A very high proportion of the total cross-sectional area of the stem tissues was now lignified.

The lignin determinations reflected this relationship. Figure 1 shows that a very low lignin concentration was present in both cultivated grasses, Stage 1. With advancing maturity a rapid rise in percentage occurred and and the steep slope of the curves indicated a similar trend for both the brome and crested wheatgrass. These histological observations on the progress of lignification in the cultivated grasses are similar to those reported by Drapala *et al.* (9) for red clover.

An interesting relationship between the number of vascular bundles per unit leaf area and lignin content was indicated by the extremely high lignin percentage, 9.6 per cent for sandgrass at Stage 1. Figure 15 shows that the vascular bundles were at least twice as numerous per unit area in this species of grass leaf than was the case for crested wheatgrass (Figure 13), which had a lignin content of only 5.5 per cent at Stage 1.

Cellulose

The success of the curing habit depends basically on the inherent ability of the aerial parts of the plant to resist deterioration over a period of several months following the curing process. The anatomy of the basal leaf of the native grasses (Figures 5, 6, 7, and 8), suggests that reinforcing fibres play a major role in the observed ability of these leaves to retain their physical form after curing occurs. These fibres formed, in most cases, a complete band several fibres wide around the inside of the lower epidermis; fescue had a heavier band than speargrass. In addition, the vascular bundles were strongly reinforced by fibres which extended above and below in the form of "I" beams. The total cross-sectional area occupied by the fibres appeared to be somewhat greater in the case of fescue than in that of speargrass (Figures 6 and 8). This observation agreed with the somewhat higher cellulose values reported for fescue leaf than those for speargrass (Figure 2).

An entirely different picture was presented by the anatomy of the cultivated grass leaf. These leaves were wider, thinner, less compact than the native grass leaves, with only a few reinforcing cellulose strands which occurred immediately above and below the widely separated vascular

FIGURE 5. Stage 2, R. Fescue.

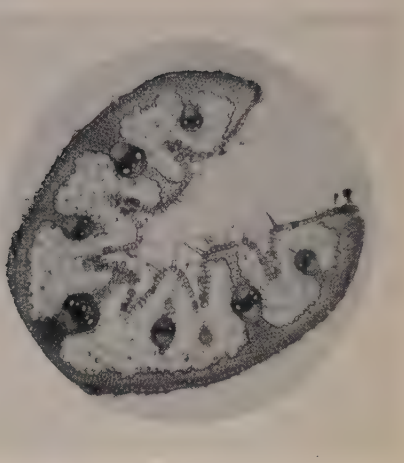


FIGURE 6. Stage 4, R. Fescue.

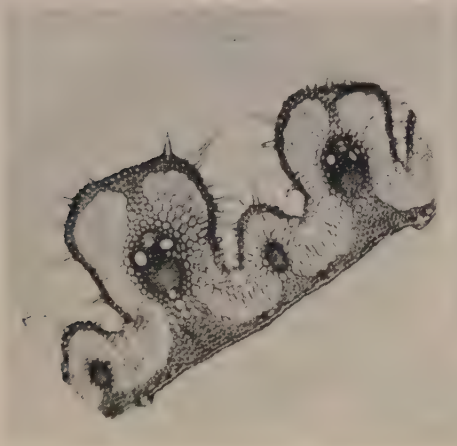


FIGURE 7. Stage 2, C. Speargrass.

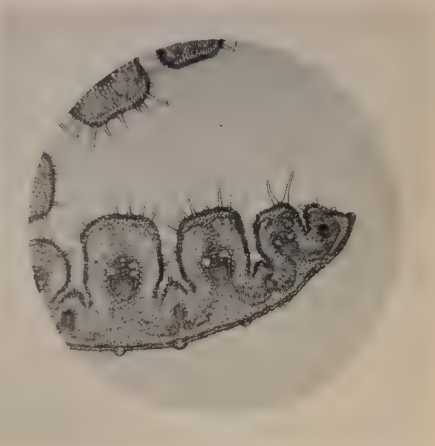


FIGURE 8. Stage 4, C. Speargrass.

Cross-Sections of Basal Leaves

FIGURE 5. Fibre development well advanced. Lignification confined chiefly to vascular bundles with fibre walls slightly lignified. Note fibre layers under lower epidermis.

FIGURE 6. Fibre development completed; fibres still only slightly lignified. No lignin in mesophyll cell walls. Numerous fat globules clearly visible in mesophyll cytoplasm.

FIGURE 7. Fibre development not yet complete. Lignification confined to vascular bundles.

FIGURE 8. Fibre development complete. Some lignification in fibres, especially below vascular bundles, but none in mesophyll cell walls. Fat globules visible in mesophyll cells.

(Magnifications × 65)

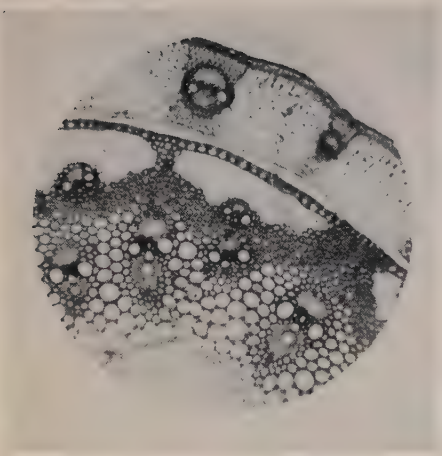


FIGURE 9. Stage 2, C. Wheatgrass.

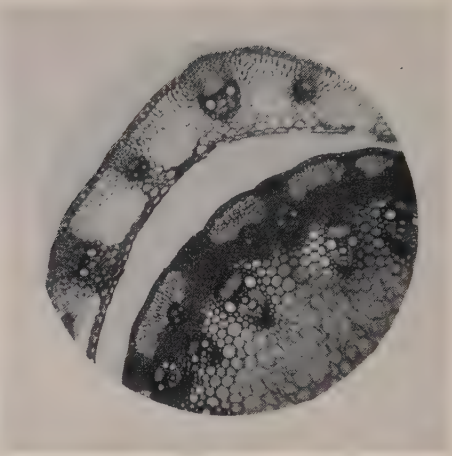


FIGURE 10. Stage 4, C. Wheatgrass.

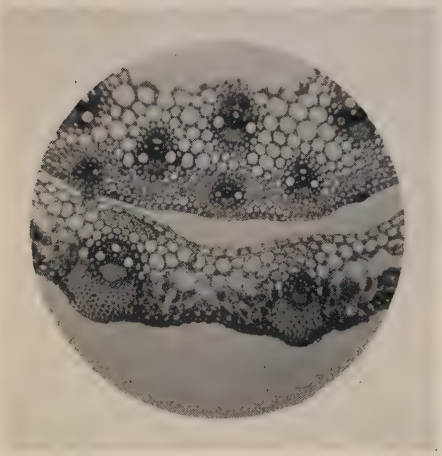


FIGURE 11. Stage 2, S. Bromegrass.

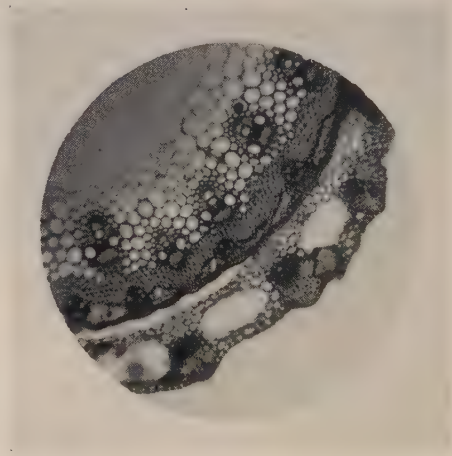


FIGURE 12. Stage 4, S. Bromegrass.

Cross-Sections of Stem and Leaf Sheaths

FIGURE 9. Slight lignification of fibres and of stem parenchyma adjacent to the bundles.

FIGURE 10. Much heavier lignification of stem parenchyma adjacent to the bundles.

FIGURE 11. Little or no lignification of fibres, but slight lignification of stem parenchyma.

FIGURE 12. Much heavier lignification of both fibres and stem parenchyma and disintegration of mesophyll in the leaf sheath.

(Magnifications × 65)

bundles (Figure 13). The brome leaf (not illustrated) was even wider and thinner as well as being structurally weaker than that of crested wheatgrass. Hence, the cultivated grass leaves were weakly reinforced and this explains why many of them, especially brome leaves, tend to decompose soon after they mature in the fall, frequently as early as mid-September.

Figure 14 shows that speargrass leaves retained their general form even after long periods of weathering, apparently due to the heavy fibre development.

Additional information on the importance of structural fibres in the leaf was obtained from the two sub-dominants on the speargrass site, namely, sandgrass and ricegrass. Cross-sections obtained from both grasses, including both the early growth and the previous year's growth, are illustrated in Figures 15, 16, 17 and 18.

Sandgrass had a fairly wide, flat leaf, which from outward appearances was similar to that of crested wheatgrass. The internal structure, however, was very different, in that it had very closely spaced vascular bundles which were well reinforced above and below with strands of strong cellulose fibres (Figure 16), in marked contrast to the widely spaced, poorly reinforced bundles of the crested wheatgrass leaf (Figure 13). Although some of the soft internal structures of the sandgrass leaf were obviously breaking up (Figure 16), the old leaf was still in an excellent state of preservation with form and structure well maintained.

It should be mentioned here that this grass is of little value for pasture as it becomes very tough and unpalatable to livestock as it matures. Nevertheless, it serves to illustrate the importance of leaf structure in relation to the curing property.

The ricegrass is closely related to speargrass taxonomically (38) and the leaf structures are similar; compare Figures 17 and 8. The old leaf, Stage 5 (Figure 18) shows that the vascular bundles and reinforcing fibres were essentially intact, enabling the leaf to retain its physical form although the softer tissues had largely degenerated. This grass provides palatable nutritious forage, but it is of little economic importance in the area because of its relative scarcity.

Figure 2 shows that a much higher proportion of the total cellulose was present in the native grass leaves at early growth stages than was the case for the cultivated grasses. Hence, the results of the dicromate method of isolating cellulose agreed well with the histological observations on the progressive formation of fibres in both native and cultivated grasses.

Ether Extract

Figure 3 indicates that the ether extract of both cultivated grasses followed the usual downward trend during the season, while that of speargrass remained nearly constant and the values for fescue steadily increased. The microchemical test for fat indicated that much of this increase was probably due to globules which gave a positive stain with Sudan IV and which reached substantial proportions, particularly in the cells of fescue leaf (Figure 6), and to a lesser extent in speargrass leaf (Figure 8). Presumably these are elaioplasts which appear to be centres of fat formation in certain plant species (29).

As previously mentioned, most of the ether extracts of forages are of low nutritional value. In this instance, Stage 4, the ether extracts of the native grass leaves were not highly coloured, hence little chlorophyll was included. Furthermore, the percentage ether extracts of native grass leaves, Stage 5, was relatively high, indicating that this characteristic was not confined to a single season. These observations suggested that probably much of the crude fat obtained from the cured nature grasses was actually esters of fatty acids, and since true fats have two and one-quarter times the energy value of carbohydrates, would provide a small but valuable source of energy to the range animal.

Crude Protein

Figure 4 shows that there was a steady decline in protein content of all four grasses throughout the season. Speargrass was noticeably higher in protein than were the other three in the cured state, with crested wheatgrass intermediate and the other two very low. If protein is one of the limiting factors in the digestibility of these roughages, as it may be, then speargrass and crested wheatgrass were sufficiently above the other two to make some difference in nutritive value. According to Clarke and Tisdale (3) there seems to be little or no indication of a higher crude protein content in the case of forages that cure well compared to those which do not cure.

Starch

There was no evidence of appreciable amounts of starch at the cured stage in any of the four grasses. Most samples gave a negative test and none exceeded the one per cent point.

DISCUSSION

Owing to drought, growth was very poor on the speargrass, fescue and crested wheatgrass sites during the 1949 season. The bromegrass site was located in a more favourable position and growth on this area was nearly normal. In addition, some of the late fescue growth was frozen before curing occurred. Whether drought or frost affected the results of the chemical analyses is not known.

The values reported for the lignin analyses of the two cultivated grasses, Stage 4, are considerably lower than those reported for the corresponding grass species in Montana (34, 35). Since the so-called formalin procedure which was used by these workers has been shown to give higher lignin values for mature plant material than does the procedure used in this study (13), the differences are probably due to this factor.

The lignin values obtained for speargrass and fescue leaves, Stage 5, are worthy of special comment. They were almost identical with those obtained for Stage 4. Unless the initial lignin content of these grasses in 1948 was much lower than that of 1949, little loss of soluble constituents has occurred; otherwise the percentage of lignin would have increased.

Whether the percentage increases of cellulose and lignin in the cultivated grasses, especially brome, between Stages 3 and 4, are really due to actual increases in these two constituents or are only apparent increases caused by losses of the more soluble carbohydrates, is not known, since analyses for these fractions were not carried out.

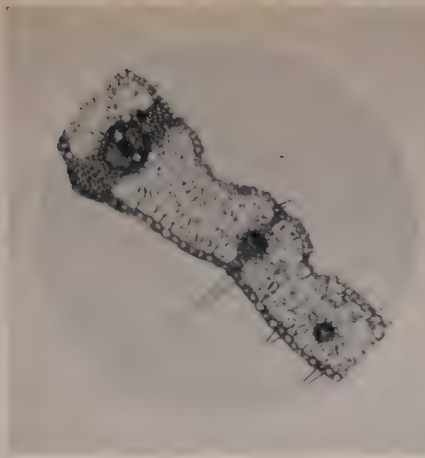


FIGURE 13. Stage 2, C. Wheatgrass.

FIGURE 14. Stage 5, C. Speargrass.

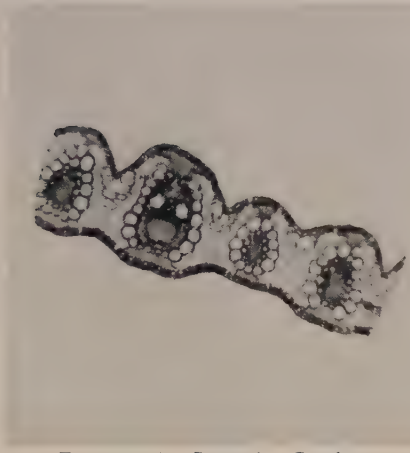


FIGURE 15. Stage 2. Sandgrass.

FIGURE 16. Stage 5. Sandgrass.

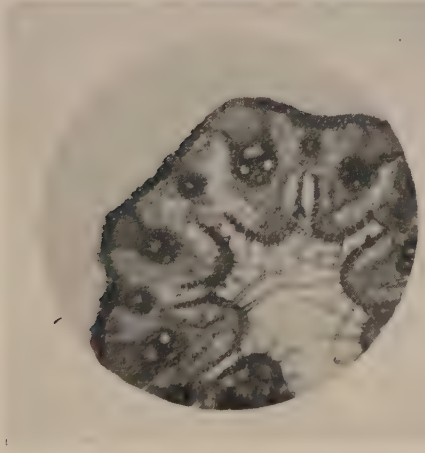


FIGURE 17. Stage 2. Ricegrass.

FIGURE 18. Stage 5. Ricegrass.

FIGURE 13. Widely spaced vascular bundles with few reinforcing fibres.

FIGURE 14. Weathered leaf showing deterioration of mesophyll, but form and shape well preserved due to reinforcing fibres.

FIGURE 15. Vascular bundles closely spaced, fibre development not yet complete.

FIGURE 16. Weathered leaf, form and shape well preserved due to strong fibre reinforcement.

FIGURE 17. Compact leaf, similar in structure to speargrass, Figure 6.

FIGURE 18. Weathered leaf, mesophyll deteriorated but numerous fibres keep general form and structure intact.

(Magnifications × 65)

The importance of lignification to curing is believed to be not necessarily total lignin content of the forage, but rather the position and extent of the lignified tissues. A large proportion of the native grass leaf, Stage 4, consisted of non-lignified mesophyll tissue, of which both the cell walls and cell contents would probably be readily digested by ruminants. Also, in general, the fibres were not heavily lignified and hence the cellulose in their walls should be quite susceptible to bacterial attack and decomposition in the digestive tract of herbivora.

Conversely, a very high proportion of the stem material of the cultivated grasses is structural material, most of which was heavily lignified at Stage 4. This material would probably be digested to a lesser degree than the material in the native grass leaves and would be expected to provide the animal with less energy.

Further studies in conjunction with animal digestion trials are desirable before definite conclusions can be drawn.

ACKNOWLEDGMENTS

The author gratefully acknowledges guidance and advice from A. G. McCalla, Dean, Faculty of Agriculture, University of Alberta, during these investigations.

Special thanks are due to J. B. Campbell, Officer-in-Charge, Pasture Division, Dominion Experimental Station, Swift Current, Sask., who originally suggested the problem, arranged for facilities to carry out much of the work at Swift Current, and gave much encouragement and many helpful suggestions.

Permission to use laboratory facilities in the Soil Research Laboratory at Swift Current was kindly granted by J. L. Doughty, Officer-in-Charge. Technical advice in handling equipment by F. G. Warder, Agricultural Research Officer, is much appreciated.

Generous financial assistance was supplied by Canada Packers Limited in the form of the "J. S. McLean Scholarship for Grassland Investigations".

REFERENCES

1. Burroughs, W., P. Gerlaugh, B. H. Edgington, and R. M. Bethke. Further observations on the effect of proteins upon roughage digestion in cattle. J.An.Sci.8 : 9-18. 1949.
2. Clarke, S. E., and D. H. Heinrichs. Regrassing abandoned farms, submarginal cultivated lands and depleted pastures in the prairie areas of western Canada. Dom. of Can. Dept. Agr. Farmers' Bull. 103. 1941.
3. Clarke, S. E., and E. W. Tisdale. The chemical composition of native forage plants of southern Alberta and Saskatchewan in relation to grazing practices. Dom. of Can. Dept. Agr. Tech. Bull 54. 1945.
4. Crampton, E. W. Pasture studies. XIV. The nutritive value of pasture herbage. Sci. Agr. 19 : 345-357. 1939.
5. Crampton, E. W., and R. P. Forshaw. Pasture studies. XV. The intraseasonal changes in the nutritive value of pasture herbage. Sci. Agr. 19 : 701-711. 1939.
6. Crampton, E. W., and L. A. Maynard. The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutr. 15 : 283-395. 1938.
7. Davies, A. W., R. A. Evans, and W. C. Evans. Studies on the biochemistry of pasture plants. 1. A new technique for the preparation and preservation of herbage samples. J. Brit. Grassl. Soc. 3 : 153-158. 1948.
8. Dexter, S. T. The yield and sugar content of alfalfa cut at various times of the day and the sugar content of the hay after various methods of drying. J. Am. Soc. Agron. 37 : 394-399. 1945.
9. Drapala, W. J., L. G. Raymond, and E. W. Crampton. Pasture studies. XXVII. The effects of maturity of the plant and its lignification and subsequent digestibility by animals as indicated by methods of plant histology. Sci. Agr. 27 : 36-41. 1947.

10. Druce, E., and J. S. Willcox. The determination of cellulose in nutritional studies. Part I. A new method for the determination of cellulose. J. Agr. Sci. 39 : 145-152. 1949.
11. Ekelund, S. Carbohydrates in hay. Ann. Royal Agr. Coll. Sweden 16 : 1-149. 1949.
12. Ellis, G. H. Report on lignin and cellulose in plants. J. Assoc. Off. Agr. Chem. 32 : 287-291. 1949.
13. Ellis, G. H., G. Matrone, and L. A. Maynard. Seventy-two per cent sulphuric acid method for the determination of lignin and its use in animal nutrition studies. J. An. Sci. 5 : 285-297. 1946.
14. Ferguson, W. S. Digestibility of wheat straw and wheat straw pulp. Biochem. J. 36 : 786-791. 1942.
15. Forbes, R. M., and W. P. Garrigus. Application of a lignin ratio technique to the determination of the nutrient intake of grazing animals. J. An. Sci. 7 : 373-382. 1948.
16. Guilbert, H. R., and S. W. Mead. The digestibility of Bur Clover as affected by exposure to sunlight and rain. Hilgardia 6 : 1-12. 1931.
17. Male, E. B., C. W. Duncan, and C. F. Huffman. Rumen digestion in the bovine with some observations on the digestibility of alfalfa hay. J. Dairy Sci. 23 : 953-967. 1940.
18. Johannson, D. A. Plant microtechnique. 1st ed. McGraw Hill Book Co., New York. 1940.
19. Kirk, L. E., T. M. Stevenson, and S. E. Clarke. Crested Wheat Grass. Dom. of Can. Dept. Agr. Farmers' Bull. 44. 1937.
20. Lancaster, R. J. Metabolism trials with New Zealand feeding-stuffs. IV. The relative significance of lignin, cellulose, and crude fibre in the evaluation of foods. New Zeal. J. Sci. & Tech. 25A : 137-151. 1943.
21. LeClerc, J. A., and J. F. Breazeale. Plant food removed from growing plants by rain or dew. U.S.D.A. Yearbook, pp. 389-402. 1908.
22. Louw, J. G. The relative digestibility of the constituents of the carbohydrate complex of grasses at successive stages of growth with reference to their partition into crude fibre and nitrogen free extract according to the standard method for feeding stuff analysis. Onderstepoort J. Vet. Sci. and An. Ind. 17 : 165-179. 1941.
23. Louw, J. G., S. I. Bodenstein, and J. I. Quinn. Digestibility for sheep of the cellulose in a poor veld hay as affected by supplements of mixture of concentrates and green feed. Onderstepoort J. of Vet. Sci. and An. Ind. 23 : 239-260. 1948.
24. MacDougall, D., and W. A. DeLong. The effect of initial drying temperature on the apparent lignin content of plant tissues. Can. J. Res. (*B*) 20 : 40-48. 1942.
25. McAnnally, R. A. Digestion of straw by the ruminant. Biochem. J. 36 : 392-399. 1942.
26. Matrone, G., C. H. Ellis, and L. A. Maynard. A modified Norman Jenkins' method for the determination of cellulose and its use in the evaluation of feed stuffs. J. An. Sci. 5 : 306-312. 1946.
27. Maynard, L. A. Interpretation of variations in plant composition in relation to feeding value. J. Am. Soc. Agron. 29 : 504-511. 1937.
28. Methods of Analysis. Assoc. Off. Agr. Chem. 6th. ed. 1945.
29. Meyer, B. S., and D. B. Anderson. Plant physiology. D. Van Nostrand Co. Inc., New York. 1939.
30. Nordfeldt, S., O. Svanberg, and O. Claesson. Studies regarding the analysis of crude fibre. Acta Ag. Suecana 111 : 135-177. 1949.
31. Norman, A. G. The composition of crude fibre. J. Agr. Sci. 25 : 529-540. 1935.
32. Norman, A. G. A biochemical approach to grass problems. J. Am. Soc. Agron. 31 : 751-760. 1939.
33. Norman, A. G., and W. H. Fuller. Cellulose decomposition By micro-organisms. Advances in enzymology and related subjects, 2 : 239-264. Interscience Pub. Inc., New York. 1942.
34. Patton, A. R. Seasonal changes in the lignin and cellulose content of some Montana grasses. J. An. Sci. 2 : 59-62. 1943.
35. Patton, A. R., and L. Gieseker. Seasonal changes in the lignin and cellulose content of some Montana grasses. J. An. Sci. 1 : 22-26. 1942.
36. Phillips, T. G., and M. E. Loughlin. Composition and digestible energy of hays fed to cattle. J. Agr. Res. 78 : 389-395. 1949.
37. Rapp, K. E. Carbohydrate metabolism of Johnson grass. J. Am. Soc. Agron. 39 : 869-873. 1947.
38. Rydberg, P. A. Flora of the Rocky Mountains and adjacent plains. Steinman and Foltz, Lancaster, Pa., New York. 1918.
39. Scales, F. M., and A. P. Harrison. Boric acid modification of the Kjeldahl method for crop and soil analysis. Ind. and Eng. Chem. 12 : 350-352. 1920.

FURTHER EXPERIMENTS WITH DDT IN THE CONTROL OF *SIMULIUM ARCTICUM* MALL. IN THE NORTH AND SOUTH SASKATCHEWAN RIVERS[1]

F. J. H. Fredeen[2], A. P. Arnason[3], B. Berck[4], and J. G. Rempel[5]

[Received for publication December 9, 1952]

ABSTRACT

In 6 tests during the period 1949 to 1951 single 15-minute applications of 0.1 p.p.m. of DDT practically eliminated black-fly larvae (*Simulium arcticum* Mall.) from the Saskatchewan River for distances up to 115 miles. Best results were obtained when the water was turbid with finely divided inorganic material. Fish were killed during 2 tests in 1949, apparently because the larvicide used was heavier than water and settled as globules on the river bed and was ingested. In laboratory tests with a DDT solution with a specific gravity of less than 1.0, fish were not affected by immersion for 3 hours in water containing as much as 100 p.p.m. of DDT. The larvicide affected black-fly larvae more than other aquatic insects. In 2 tests black-fly larvae were eliminated without apparent effect on other aquatic organisms.

DDT was extracted from suspended solids from water samples collected as far as 68 miles downstream from the point of application. At this distance, 2.26 to 0.24 μg. of DDT per gram of suspended solids was extracted, equivalent to 1 part of DDT in 685 to 4,385 million parts of water. Black-fly larvae ingest particles suspended in water. This suggests that the effectiveness for long distances of single applications of the larvicide in turbid water is due to ingestion of DDT adsorbed by solids rather than from contact with it. Other aquatic larvae suffered lower mortality from the treatment, apparently because they did not ingest suspended matter.

INTRODUCTION

The results reported in this paper are from investigations conducted in 1949, 1950, and 1951 on the use of DDT as a larvicide for the control of black flies in the Saskatchewan River. Preliminary tests in 1948 (1) demonstrated that an oil solution of DDT, applied from an aircraft to give a dosage at the point of application of 0.13 p.p.m. of DDT for 36 minutes, eliminated black-fly larvae for 17 miles. There was good evidence that the larvicide was highly effective for some 70 miles farther downstream. Aquatic insects other than black-fly larvae were affected to a lesser degree and fish were unharmed.

The results of the 1948 investigation and of studies in northern Manitoba (6, 7, 10, 11), the Yukon (7), Kenya Colony (4), and Guatemala (3) were so promising that field tests for the development of a practical program of black-fly control for the Saskatchewan River were continued in 1949, 1950, and 1951. A major objective was to obtain information on the length of stream that could be freed of black-fly larvae by a single application of DDT. The uniform rate of flow and width of river for more than 100 miles provided an opportunity to obtain information on the distribution and dilution of DDT as it passed downstream. Data were also obtained on the relationship between DDT and solids suspended in the river water.

[1] Joint contribution, Division of Entomology (No. 3038), Science Service, Department of Agriculture, Ottawa, Canada, and Department of Biology, University of Saskatchewan, Saskatoon.

[2] Agricultural Research Officer, Veterinary and Medical Entomology Unit, Laboratory of Entomology, Saskatoon.

[3] Officer-in-Charge, Laboratory of Entomology, Saskatoon.

[4] Chemist, Stored Product Insect Unit, Laboratory of Entomology, Winnipeg, Man.

[5] Professor, Department of Biology, University of Saskatchewan.

TABLE 1.—DATA FROM 6 FIELD TESTS ON THE CONTROL OF *Simulium arcticum* IN THE NORTH AND SOUTH SASKATCHEWAN RIVERS WITH DDT

Test	Date and point of application	River discharge[1] (cu. ft./sec.)	Suspended solids content[2] (p.p.m.)	Water temp. (° F.)	Water pH[2]	Nature of larvicide[3]	Application			
							Method	Amount (gal.)	Duration (min.)	Rate (p.p.m.)
1949A	May 10, Clarkboro, South Saskatchewan	5,700	247	57	8.5	DDT concentrate[4]	15 5-gal. cans, 50′ apart on bridge	64	24	0.39
1949B	June 24, Saskatoon, South Saskatchewan	12,200	166	64	8.4	DDT concentrate[4]	8 5-gal. cans 90′ apart on bridge	26.4	16	0.11
1950A	May 29, Saskatoon, South Saskatchewan	16,400	551	58	8.2	DDT concentrate[4] diluted with kerosene to give 10% solution, sp. gr. = 0.9	3 45-gal. drums, 200′ apart on bridge	90	16	0.089
1950B	July 30, Clarkboro, South Saskatchewan	14,000	183	—	—	DDT concentrate[4] diluted with kerosene to give 10% solution, sp. gr. = 0.9	4 45-gal. drums on ferry	120	16	0.115
1951A	May 22, Saskatoon, South Saskatchewan	44,000	1,068	59	—	DDT concentrate[4] diluted with kerosene to give 10% solution, sp. gr. = 0.9	6 45-gal. drums on bridge	275	17	0.09
1951B	May 25, Crutwell, North Saskatchewan	16,500	374	57	—	DDT concentrate[4] diluted with kerosene to give 10% solution, sp. gr. = 0.9	3 45-gal. drums on ferry	111	12	0.114

[1] Dominion Water and Power Bureau, Saskatchewan River Records.
[2] Determined by Department of Chemistry, University of Saskatchewan.
[3] Concentrate purchased from Charles Albert Smith Ltd., Toronto, Ont
[4] Thirty per cent w/w DDT in methylated naphthalene, sp. gr. = 1.04.

FIGURE. 1. Map of the North and South Saskatchewan Rivers showing points of applications of insecticide against black-fly larvae, and the locations of rapids sampled for larvae.

MATERIALS, METHODS, AND PROCEDURE

Six tests were conducted on the North and South Saskatchewan Rivers (Figure 1), two in each of the years 1949, 1950, and 1951. Table 1 shows some of the data from these tests. Supplementary tests were made to study the effects of DDT on fish.

Test 1949A

The larvicide was dispensed from containers fitted with short lengths of oil-resistant tubing. The rate of flow of larvicide was controlled with screw clamps. At a depth of 3 feet, samples were taken 3 miles downstream from the point of application as the treated water passed that point. DDT content was determined by the Schechter-Haller (9) method, and effects on black-fly larvae were correlated with observations made before and after treatment at 10 rapids along 162 miles of the river. No observations were made at that time of the insect fauna of mud or of sand beds in the river. Fish found dead were frozen in dry ice and expressed to the Laboratory of Entomology, Winnipeg, for determination of DDT content.

Test 1949 B

The effects of this application on black-fly larvae and other insects were determined quantitatively at four rapids for a distance of 60 miles (Table 3). The method of estimating populations was essentially identical with that used in 1948 (1).

Test 1950A

Quantitative samples of black-fly larvae and other aquatic insects were collected before and after the application from rapids as far as 115 miles

TABLE 2.—DDT CONTENT OF RIVER WATER, 3 MILES DOWNSTREAM FROM THE POINT OF APPLICATION, TEST 1949A

Source	Time	DDT recovered (p.p.m.)	DDT : water ratio[1]
Forward zone, midstream	11.34	0.0043	$1 : 184 \times 10^6$
Central zone, midstream	11.39	0.0043	$1 : 184 \times 10^6$
Central zone, east bank	11.46	0.0048	$1 : 167 \times 10^6$
Central zone, west bank	11.53	0.0055	$1 : 145 \times 10^6$
Rear zone, midstream	11.59	0.0035	$1 : 227 \times 10^6$
Rear zone, east bank	12.05	0.0053	$1 : 154 \times 10^6$
Control, sampled before arrival of treated water	11.25	0.00	—
Oil film first observed	11.32	—	—
Oil film last observed	12.12	—	—

[1] Calculated on the basis of 80 per cent average recovery from DDT river water standards (2).

TABLE 3.—EFFECTS OF DDT LARVICIDE ON BLACK-FLY LARVAE AND OTHER AQUATIC INSECTS IN THE SOUTH SASKATCHEWAN RIVER, TEST 1949B

Sampling site and distance in miles downstream from point of application	Insects per sq. ft., June 23 and 24		Insects per sq. ft., June 29		Per cent reduction	
	Simuliid larvae	Other insects	Simuliid larvae	Other insects	Simuliid larvae	Other insects
1. Clarkboro, 17	10	11.2	0.8	4.4	92	61
2. Hague, 35	370	52.0	5.0	21.0	99	60
3. Fish Creek, 41	304	29.0	4.8	7.0	98	76
4. Batoche, 59	358	33.0	128.0	28.0	64	15

from the point of application (Table 6). These collections were supplemented in every case by counting in unit areas of marked colonies with the aid of a viewing glass.

An attempt was made, particularly in 1950, to determine both the distribution of DDT in the river and the rate of dilution, in association with observations of toxic effects on black-fly larvae. Samples of river water and suspended solids were taken at predetermined sites. The water samples varied in size from 2 quarts to 4 gallons (U.S.), according to the distance from the point of application. Samples were taken from 1 to 6 inches below the surface and from 1 to 2 feet above the river bottom near the east bank, the centre, and the west bank at five stations situated 5, 17, 35, 52, and 68 miles downstream. At each station samples were taken before, during, and after the calculated passage of the treated water. Suspended solids were collected at the 17-mile station in specially constructed cotton bags. These were anchored in the stream for 20 minutes during the caculated period of passage of the treated water. At the 52- and 68-mile stations, suspended solids were extracted from 21 gallons of water. Details of sampling and of analysis of the DDT content of the

TABLE 4.—DDT CONTENT OF SASKATCHEWAN RIVER WATER, TEST 1950A

Distance downstream from point of application and time of sampling	Samples collected	DDT : water ($\times 10^6$) ratio
5 miles—		
A. 5.45 p.m. (calculated first appearance)	2	1 : 77 and 1 : 100
B. 6.00 p.m.	6	1 : 39 to 1 : 134
C. 6.15 p.m.	3	1 : 75 to 1 : 105
D. 6.30 p.m. (calculated near edge of treated water)	3	1 : 104 to 1 : 114
Average of 14 samples		1 : 82
17 miles—		
A. 8.20 p.m.	1	1 : 260
B. 8.53-8.55 p.m.	2	1 : 209 and 1 : 260
C. 9.05-9.11 p.m.	3	1 : 134 to 1 : 260
D. 9.28-9.31 p.m.	2	1 : 156 and 1 : 214
E. 9.38-9.44 p.m.	3	1 : 138 to 1 : 234
F. 9.56-9.59 p.m.	2	1 : 144 and 1 : 209
G. 10.19-10.35 p.m.	2	1 : 277 and 1 : 153
Average of 15 samples		1 : 195
35 miles—		
A. 12.55 a.m.	1	1 : 143
B. 1.17-1.21 a.m.	2	1 : 133 and 1 : 293
C. 1.43 a.m.	1	1 : 176
D. 2.15-2.17 a.m.	2	1 : 173 and 1 : 188
E. 2.36-2.39 a.m.	2	1 : 289 and 1 : 313
F. 2.49-2.51 a.m.	2	1 : 313 and 1 : 313
G. 2.59-3.03 a.m.	2	1 : 285 and 1 : 285
H. 3.21-3.22 a.m.	2	1 : 313 and 1 : 517
I. 4.05-4.06 a.m.	2	1 : 517 and 1 : 517
Average of 16 samples		1 : 298

river water and suspended solids are presented elsewhere (2). A modification of the Schechter-Haller spectrophotometric method (9) was used in the procedure. In the analysis of samples containing sub-optimal amounts of DDT, standard addition was used*.

Test 1950B

As in the previous test the effects on aquatic insects were determined by means of quantitative samples from rapids (Table 8) and by observation and counts with the aid of a viewing glass.

To determine the amount of DDT associated with the suspended solids of the river water, four 8-gallon samples of river water were taken at Hague Ferry, 18 miles downstream from Clarkboro, beginning 3 hours after the application at Clarkboro. The suspended solids were separated by sedimentation from acidified water, and the DDT present was determined as before (2).

* Experiments on recovery of DDT dispersed in untreated water (2) showed that the range of greatest accuracy was from 4 to 30 μg. of DDT. Although 1 μg. of DDT may readily be detected with the method used, the range 1-4 μg. was considered quantitatively suboptimal. Where suboptimal DDT loads were encountered in field samples of either water or suspended solids, 10 μg. of DDT were added in the final stage of the determination (standard addition). The correction curve of the optimal 4-30 μg. range as plotted on logarithmic probability paper gives a reasonably straight line, and since this line may be extrapolated, correction factors based on mathematical probability were derived for suboptimal loads. By application of the appropriate factor, the DDT load "as found" was equated to a theoretical 100 per cent recovery. In this way, sub-optimal loads provided useful approximations of concentrations existing at the 52- and 68-mile stations. In passing, note should be taken that when standard addition is used in the foregoing manner relative analysis error is increased (8) (9).

TABLE 5.—DDT CONTENT OF SUSPENDED SOLIDS, TEST 1950A

Distance in miles downstream from point of application	Weight of solids collected gm.	Volume of water sample U.S. gal.	DDT content of solids p.p.m.	Calculated DDT : water ($\times 10^6$) ratio
A. 17	28.175 (from fabric silt collector)		1.520[1]	1 : 1,280
B. 17	27.941 (from fabric silt collector)		1.430[1]	1 : 1,350
C. 52	10.048	5	0.796	1 : 1,740
D. 52 and 68	8.467	4	0.473	1 : 2,495
E. 68	8.510	4	0.294	1 : 3,625
F. 68	8.394	4	2.261[2]	1 : 685
G. 68	8.257	4	0.242	1 : 4,385

[1] Samples A and B consisted of 14.5 per cent clay, 17.9 per cent silt, and 67.6 per cent fine sand (University of Saskatchewan, Soils Department).
[2] No explanation can be advanced for the relatively high DDT load in sample F.

Tests 1951A and B

These were made as an additional check on the effectiveness of 10 per cent w/w methylated naphthalene and kerosene solution of DDT as a black-fly larvicide in both branches of the Saskatchewan River.

Supplementary Tests

Forty-three tests were made to determine the effect on fish of contact with DDT in solution and suspension in water. A total of 394 suckers and chubs (*Moxostoma aureolum* (Le Sueur) and *Platygobio gracillis* (Rich.)) from the Saskatchewan River were exposed for 15 minutes to 3 hours to the following concentrations in a 100-gallon tank: 5 to 30 p.p.m. of DDT as a 10 per cent solution in a 9 : 1 mixture of fuel oil and Velsicol AR 50, 1 to 25 p.p.m. of DDT as a 50 per cent wettable powder, and 0.3 to 100 p.p.m. of DDT applied as J.P. 30. Also 227 river minnows (*Notropis blennius* (Gir.) and *Pimephales promelas* Raf.) were exposed for 30 minutes to 1 to 50 p.p.m. of technical grade DDT. Following each test the fish were held in observation cages in the river for 2 to 3 days.

In addition, applications of J.P. 30, the larvicide used in tests 1949A and B, were made to two shallow rivers, the Waskesiu and Montreal Rivers in northern Saskatchewan, to study the dispersion of the material in water and its effect on fish. Suckers of the same species as those found in the Saskatchewan River were placed in an enclosure over deposits of the 30 per cent DDT concentrate accumulated on the river-bed.

To determine the effect of feeding DDT-killed insects to fish two northern suckers were fed approximately 1 per cent of their body weight of aquatic insects that had been killed by a 1-hour exposure to 2 p.p.m. of DDT.

BIOLOGICAL AND PHYSICAL DATA

Black-fly larvae were relatively scarce in the Saskatchewan River in 1949, 1950, and 1951 as compared with the preceding years. In 1949,

TABLE 6.—EFFECTS OF DDT LARVICIDE ON BLACK-FLY LARVAE AND OTHER AQUATIC INSECTS IN THE SOUTH SASKATCHEWAN RIVER, TEST 1950A

Sampling site and distance in miles downstream from point of application	Total insects from 33 sq. ft.				Calculated reduction of simuliid population, per cent
	May 26		May 31 and June 1		
	Simuliid larvae	Other insects	Simuliid larvae	Other insects	
1. Sutherland, 2	53	1	0	3	100
2. Clarkboro, 17[1]	22	8	135	7	—
3. Hague, 35	613	2	11	8	98
4. Fish Creek, 41	282	20	10	11	96
5. Batoche, 59	120	6	23	13	81
6. Fenton, 97	1,592	22	25	33	99
7. Winton, 115	66,000	—	165	—	99

[1] Samples collected from rapids along west bank. Larvicide was apparently concentrated along the east bank, at least as far downstream as this station, as a result of strong westerly winds.

hatching commenced on April 14, less than a week after the ice left the river. Second-instar larvae were present on April 22, and at the time of the first application on May 10 a few larvae were nearly full-grown. During the second application, larvae of all instars and pupae were present in the rapids. The river level during each application was lower than normal for the period, and the water was relatively free of suspended solids. This was in marked contrast with the unusually high river level in 1948 and the substantial amounts of suspended solids carried by the river in 1948, 1950, and 1951.

In 1950, hatching commenced on May 1. The larvae developed slowly and were only half-grown by May 23. The rapids became re-infested after the first application, and on July 30 larvae of all instars and pupae were present.

In 1951, hatching commenced on April 28 and pupation on May 28, 6 days after application of the larvicide.

RESULTS

Test 1949A

Water samples collected 3 miles downstream from the point of application at a depth of 3 feet contained 1 part of DDT in 145 million of water to 1 part in 227 million (Table 2). The concentration of DDT at this point was 0.009 to 0.014 of the amount applied, and 0.06 to 0.35 of the concentrations recovered in the effective test of 1948 (1).

The larvicide, applied at intervals of 50 feet across the width of the river, formed oil films on the water surface. These united into a single broad sheet that covered the entire river at a point one-half mile downstream from the point of application.

Observations at rapids infested with black-fly larvae indicated that the treatment destroyed almost 100 per cent of the larvae for at least 39 miles. However, the lethal effect apparently extended farther for small numbers of dead black-fly larvae were found attached to rocks at Fenton, 80 miles downstream from the point of application. Nevertheless, larvae

were apparently still present in sufficient numbers in the Fenton area to contribute to an outbreak on June 5 that resulted in the death of 12 head of livestock in the Melfort district, 30 miles east of Fenton.

Surveys of adult populations in June confirmed the results of surveys of larval populations. Black flies attacked livestock in the vicinity of Saskatoon, 17 miles upstream from the point of application, and in the Fenton area, 80 miles downstream, but were practically absent in the intervening area.

It was estimated that more than half of the aquatic insects other than black-fly larvae were also eliminated within the treated area.

The DDT treatment proved lethal to river fish. A single disabled fish was seen at the Hague rapids about 17 hours after the larvicide had passed that point. A few hours later the first dead fish were found in that area, and for several hours dead and disabled fish appeared along a 60-mile length of the river. A careful census indicated that along this 60-mile stretch of the river 13,000 fish were dead, or an average of 2 fish per acre. The largest numbers of dead fish were found in an area centring 25 miles downstream from the application point at Clarkboro. The change in fish population of the river was not known, but fishermen along this section of the river observed no change in catch during May and June, suggesting that only a small portion of the fish had been killed.

Of the fish found dead, 64 per cent belonged to three species of sucker, *Moxostoma aureolum* (Le Sueur), *Catostomus commersonii* (Lacepede), and *Ictiobus cyprinella* (Cuv. & Val.); 28 per cent were goldeye, *Hiodon alosoides* Raf.; and the remainder were perch, *Stizostedion vitreum* (Mitch.), and chub, *Platygobio gracilis* (Rich.). A few sturgeon, *Acipenser fulvescens* Raf., were found. No piscivorous fish, such as the northern pike, were found, although they are known to be present in the Saskatchewan River.

The stomach contents of the fish yielded as high as 157 p.p.m. of DDT for the common sucker and 919 p.p.m. for the goldeye. The liver, gills, and edible flesh contained much smaller quantities, from 1.8 to 18.2 p.p.m.

Test 1948B

The calculated DDT dose of 0.11 p.p.m. for 16 minutes was approximately one-quarter of the applied concentration and two-thirds of the duration of exposure of the first test, and is approximately the dosage that was found to be effective elsewhere (6, 7, 10) in ridding streams of black-fly larvae without affecting fish. The actual concentration of DDT in the water was not determined.

The results obtained from quantitative samples of black-fly larvae and of other aquatic insects are presented in Table 3. Populations of black-fly larvae were reduced more than 98 per cent at Fish Creek rapids, 41 miles downstream from the point of application, and 64 per cent at Batoche, 59 miles downstream. Counts of marked colonies of black-fly larvae, made with the aid of a viewing glass, confirmed these results. The initial population density of black-fly larvae at Station 1 (Table 3) was low and an accurate estimate of change at that point was not possible. In small rapids upstream from the point of application black-fly larvae were scarce, but observations made there indicated that no change in population numbers had occurred during the test.

The larvicide evidently had no lethal effect on black-fly pupae, since adult insects emerged from marked colonies within 5 days after the application. Similarly, black-fly eggs were not killed since the entire cleared section of the river became re-infested with larvae within 5 days after the application. These observations emphasize both the importance of applying DDT at the correct time in the life-cycle of the black fly, and also the necessity of systematic observation and analysis of insect populations.

The DDT application again affected other aquatic insects as well as black-fly larvae. The average numerical reduction of the former in the first 41 miles of the treated section of the river was 66 per cent. Chironomid larvae in these rapids were affected to a lesser degree than were larvae of Plecoptera, Ephemeroptera, and Trichoptera.

The treatment again was lethal to fish. Dead fish were found on the shores for 90 miles, beginning about 3 miles below the point of application. Most of these fish were found 20 to 30 miles downstream from the point of application. Counts at 11 representative places indicated that there were 10,000 dead fish or an average of 1 fish per acre.

Test 1950A

The larvicide, siphoned from 3 drums, formed 3 broad sheets of oil film on the water surface which merged farther downstream. A strong westerly wind tended to direct the oil films toward the east bank of the river. The analysis of water samples for DDT (Table 4) indicated that 5 miles downstream the average ratio was $1 : 82 \times 10^6$ throughout a sampling period of 45 minutes; at 17 miles downstream, $1 : 195 \times 10^6$ for $2\frac{1}{3}$ hours; and 35 miles downstream, $1 : 298 \times 10^6$ for $3\frac{1}{4}$ hours.

For the first time it was demonstrated that DDT was associated with the suspended solids in the water (Table 5). For example, 17 to 68 miles downstream from the point of application, the DDT content per gram of suspended solids varied from 0.242 to 2.261 μg. This was calculated to be equivalent to DDT: water ratios ranging from $1 : 4{,}385 \times 10^6$ to $1 : 685 \times 10^6$. The suspended solids collected at the 17-mile site were obtained by use of silt collectors anchored in the stream and were composed of 14.5 per cent clay, 17.9 per cent silt, and 67.6 per cent fine sand*. This material was coarser than that obtained by sedimentation from whole-water samples taken at the 52- and 68-mile sites.

Black-fly larvae were almost eliminated as far as Fenton rapids, 97 miles downstream from the point of application (Table 6). Counts of black-fly larvae in marked colonies agreed closely with counts in quantitative samples, and also indicated that black-fly larvae were almost eliminated at Winton, 115 miles from the point of application.

Quantitative sampling indicated no significant decrease in populations of other aquatic insects (Table 6). Fish were unaffected in contrast with the 1950 tests on the Saskatchewan River in which fish died as a result of the use of a 30 per cent concentrate.

Black-fly larvae were not affected in rapids along the west bank at Clarkboro, whereas along the east bank at that point and in rapids down-

* Analysed by the Soils Department, University of Saskatchewan.

TABLE 7.—DDT CONTENT OF SUSPENDED SOLIDS COLLECTED 18 MILES DOWNSTREAM FROM POINT OF APPLICATION, TEST 1950B

Quantity of water U.S. gal.	Time of collection, p.m.	DDT content of solids μg./gm.	Calculated DDT : water (× 10^6) ratio
8	5.00-5.15	0.0	—
	(3 hr. after application at Clarkboro)		
8	5.27-5.45	0.905	1 : 3,890
8	5.55-6.10	1.03	1 : 3,665
8	6.25-6.40	1.08	1 : 3,595

stream from Clarkboro the larvae were almost eliminated. Samples of river water collected near the west bank at Clarkboro contained less DDT than samples collected in the centre of the stream or near the east bank, presumably because considerable larvicide on the river surface near the point of application had been blown by strong winds towards the east bank.

Test 1950B

The larvicide, applied from barrels on a ferry in the centre of the river, spread rapidly over the water surface. One-half mile downstream from the point of application the oil film covered approximately one-third of the river surface.

DDT was extracted from suspended solids obtained by sedimentation (2) from water collected during $1\frac{1}{4}$ hours at Hague, 18 miles downstream from the point of application. The analyses (Table 7) indicate a slight increase (from 0.905 to 1.08 μg. per gram) in the DDT content of the suspended solids during the sampling period. This is equivalent to a concentration of DDT in the water of 1 : 3,870 × 10^6 to 1 : 3,595 × 10^6 because there were 183 p.p.m. of suspended solids in the water.

Quantitative samples of aquatic insects collected 1 day before and 5 days after application of the larvidide (Table 8) showed an apparent reduction in the number of black-fly larvae of 89 per cent at Hague, 82 per cent at Batoche, and 31 to 33 per cent at Fenton and Winton, respectively, which are located 18, 42, 80, and 98 miles downstream from the point of application. However, post-application samples contained first-instar larvae that had hatched after the treatment, thus reducing its apparent effectiveness. If these larvae were not included in the calculations, then less than 1 per cent of the larvae present during passage of the treated water survived at Hague and Batoche, and only 10 to 12 per cent survived at Fenton and Winton.

As in test 1950A, application of 10 per cent DDT in kerosene and methylated napthalene had no apparent harmful effects on other aquatic insects or on fish.

Black-fly larvae were eliminated from a shorter section of the river in test 1950B than in test 1950A. The outstanding difference in the conditions of these tests was the amount of suspended solids in the water: in

TABLE 8.—EFFECTS OF DDT LARVICIDE ON BLACK-FLY LARVAE AND OTHER AQUATIC INSECTS IN THE SOUTH SASKATCHEWAN RIVER, TEST 1950B

Sampling site and distance in miles from point of application	Total insects from 33 sq. ft.				Calculated reduction of simuliid population, per cent
	July 29		August 4		
	Simuliid larvae	Other insects	Simuliid larvae[1]	Other insects	
1. Clarkboro, 0.25 upstream	95	771	87	542	—
2. Hague, 18 downstream	2,350	352	258	417	89
3. Batoche, 42 downstream	234	287	41	276	82
4. Fenton, 80 downstream	170	282	118	329	31
5. Winton, 98 downstream	20,700	317	13,930	299	33

[1] This includes first-instar larvae which probably hatched after the treatment. Counts of black-fly larvae (see text) which excluded larvae that were not visible without magnification were also made and these indicated that approximately 99, 99, 90, and 88 per cent of the larvae were actually eliminated at the four points, respectively.

1950A, 551 p.p.m.; in 1950B, 183 p.p.m. A relationship between suspended solids and biological effectiveness of the larvicide treatment was thus indicated.

Test 1951A

Considerable numbers of black-fly larvae occurred in the rapids before the application, but after the application only 20 mature larvae and pupae were found on June 8 during a search of rapids at Hague, Batoche, Fenton, and Winton, 35 to 115 miles downstream from the point of application. However, a small number of early-instar larvae that apparently hatched after the application were found in all rapids. Fish were unaffected by the treatment. Black flies were not troublesome along this river in 1951.

Test 1951B

The two major infestations of black-fly larvae in the North Saskatchewan River in 1951 were at Prince Albert and Cole Falls, 18 and 48 miles, respectively, downstream from the point of application. The infestations were almost eliminated as a result of this test and apparently this entire section of the river was freed from larvae. No serious black-fly attacks occurred along this river subsequent to the treatment. Fish were unaffected.

Supplementary Tests

In tank tests no fish were disabled or killed by 3-hour exposures to as much as 100 p.p.m. of DDT (applied). In similar tests with minnows only two out of 227 died following 30-minutes exposures to as much as 50 p.p.m. of DDT (applied). These two minnows were observed to have touched the surface film of DDT-oil solution during one of the tests and the oil or the DDT or both may have caused death.

In tests with J.P. 30 on two shallow rivers some of the larvicide was observed to settle in globules to the river-bed, indicating that only a part of the concentrate contributed to a surface oil film. Globules which accumulated in depressions on the bottom were still there 10 days later. No mortality of fish was observed. Suckers kept in enclosures over accumlations of the J.P. 30 were not observed to feed during captivity but when released appeared to be quite normal.

Fish were not harmfully affected when fed on DDT-killed insects.

DISCUSSION

For 3 years black-fly larvae (*Simulium arcticum* Mall.) were almost completely eliminated from 40- to 115-mile sections of the Saskatchewan River by single applications of kerosene and methylated naphthalene solutions of DDT at rates as low as 0.089 p.p.m. of DDT for 16 minutes. The data confirm the results of an earlier test (1) on the Saskatchewan River in which a single application of DDT was apparently effective for 90 miles, although complete destruction of black-fly larvae was obtained for only 17 miles. These results are considerably better than any reported elsewhere. For example, in Guatemala (3) black-fly larvae were killed in a 6.83-mile section of a stream by the application of 0.13 p.p.m. of DDT, and in Kenya Colony (4) larvae were eliminated from sections up to 9 miles long by repeated applications of 2 p.p.m. of DDT for 30 minutes at 10- to 14-day intervals. In Alaska (5) and at Churchill (6, 7), control was reported for maximum distances ranging from 880 to 4,400 yards.

In test 1949A, water samples collected 3 miles downstream from a 24-minute application of 0.39 p.p.m. of DDT as a 30 per cent solution of DDT in methylated naphthalene yielded only 0.004 to 0.007 p.p.m. of DDT. Part of this discrepancy is attributed to the deposition of some of the larvicide on the bottom of the river. These concentrations are considerably lower than any previously proved effective for killing black-fly larvae. At the 3-mile section, DDT was present in the water for 40 minutes, indicating an increase of 1.7 times in volume of treated water. Because the river channel is uniform throughout, it is postulated that as the treated water moved downstream the volume of treated water continued to increase at approximately the same rate with a corresponding decrease in DDT concentration. Nevertheless, this treatment practically eliminated larvae for at least 39 miles, lending support to the formula (6) "concentration $\times$ time = a constant".

In test 1950A, with a 16-minute application of 0.089 p.p.m. of DDT as a 10 per cent solution in methylated naphthalene and kerosene, low DDT: water concentrations were again encountered with a duration of more than 3 hours 35 miles downstream from the point of application. Progressive dilution was offset by a corresponding increase in the volume of DDT-treated water, and of time during which DDT passed that point, and larvae were destroyed for over 100 miles downstream.

One of the important findings of these investigations is that DDT applied to the Saskatchewan River is adsorbed by solids suspended in the water. This may explain why single applications of DDT were effective in destroying black-fly larvae in long sections of the river, and may also

explain the selective action toward these larvae. Saskatchewan River water is especially turbid in early summer and, during the 1949-51 tests, the suspended solids content of the water ranged as high as 1,068 p.p.m. (May 22, 1951). Such solids, obtained by sedimentation from water samples taken as far as 68 miles downstream from a larvicide application, contained 0.24 to 2.26 μg. of DDT per gram. Laboratory experiments (2) proved that suspended solids originally present in the river water could adsorb DDT from 0.1 p.p.m. suspensions in distilled water. Similar adsorptive characteristics were demonstrated with dilute aqueous solutions of organic dyes. Therefore it is evident that these suspended solids possess surface-active properties and serve as the vehicle for carrying at least some of the DDT for long distances in the river.

The contents of the digestive tracts of larvae of *S. arcticum* indicated that the larvae are indiscriminate feeders and that, in general, they ingest all forms of suspended material, including considerable inorganic material. This helps to explain the mode of action of DDT as a black-fly larvicide in the Saskatchewan River and also how a single application of the larvicide can be effective for 100 miles or more. In contrast with the results recorded in this paper, DDT applied to streams containing relatively clear water in northern Manitoba, Yukon, and Alaska (5, 6, 7, 11) was effective for only a few hundred yards to 6 miles. Results of the two tests in 1950 on the South Saskatchewan River also indicate that the effectiveness of larvicide applications may be related to the quantity of total solids in the water. In test 1950A, the water contained 551 p.p.m. of suspended solids, and larvae were almost completely eliminated from the river for 115 miles. On the other hand, a similar application of DDT on July 30 (test 1950B), when the water contained only 183 p.p.m. of suspended solids, was effective less than 80 miles.

Data from these tests indicate that DDT applied to the turbid Saskatchewan River has a selective action. For example, in the 1950 tests, when the river was very turbid, black-fly larvae were destroyed, but other aquatic insects were not affected. In previous tests, when the river was less turbid, aquatic insects were affected but to a lesser degree than were black-fly larvae. DDT applied to clear-water streams (6, 7) showed no similar selective action. The selective action of the larvicide in the Saskatchewan River may be caused by the black-fly larvae ingesting DDT-bearing silt, whereas other aquatic insects that do not ingest such material are unaffected.

Fish were killed in tests 1949A and B in which solutions of 30 per cent DDT in methylated naphthalene were applied to the river. Only insect-feeding fish, and particularly bottom feeders, such as the sucker species, were killed. The stomach contained 50 to 100 times as much DDT as the liver, gills, and edible flesh.

Laboratory tests showed that the fish were unharmed by contact with as much as 100 p.p.m. of DDT in water for 3 hours, or by consumption of aquatic insects killed by DDT. On the other hand, in applications of the 30 per cent larvicide to shallow streams some of it settled in globules on the bottom of the streams and only a part of it was carried downstream. These results indicate that mortality of fish in the Saskatchewan River, after the

two applications of 30 per cent DDT in methylated naphthalene, was caused by the fish ingesting globules of larvicide in the water or on the river-bed.

In contrast with the results of the 1949 tests, fish were unharmed by the application to the Saskatchewan River in 1948 of 13 per cent DDT in fuel oil and Velsicol AR 50 at 0.13 and 0.07 p.p.m. for periods of 36 and 34 minutes (1), and by 4 applications in 1950 and 1951 at similar rates of DDT in 10 per cent kerosene and methylated naphthalene. The larvicide in these instances had a specific gravity of 0.9 as compared with 1.04 in the 1949 tests. Tests at Churchill, Manitoba in 1948 (10) also indicated that DDT concentrates with a specific gravity of 1.0 or less were not harmful to fish when applied to streams at concentrations effective in destroying black-fly larvae. Five per cent DDT in fuel oil applied to streams in Alaska (5) at 0.3 and 0.4 p.p.m. for 15 minutes eliminated black-fly larvae, yet rainbow trout were neither disabled nor killed by 15-minute treatments of as much as 10 p.p.m. From these results it is evident that fish mortality will not occur when kerosene or fuel oil solutions of DDT with a specific gravity of 1.0 or less are applied to streams in the small amounts required to kill black-fly larvae.

RECOMMENDATIONS

Recommendations for controlling black flies breeding in the North and South Saskatchewan rivers may be made from the results of the foregoing investigations. Elimination of the majority of larvae from their principal breeding places on the South Saskatchewan River, between Saskatoon and its confluence with the North Saskatchewan, a distance of over 100 miles, may be accomplished by 1, or possibly 2, applications of 10 per cent DDT in kerosene or fuel oil to the river, to provide a concentration of approximately 0.1 p.p.m. of DDT for 15 minutes at the point of application. The first application may be made at Saskatoon or Clarkboro, and the second, if required, at Fenton. On the North Saskatchewan River applications at Battleford and Crutwell Ferry may be required. The first applications should be made before pupation of the spring generation of larvae. To avoid toxic effects on fish, the specific gravity of the larvicide should be less than 1.0. On the basis of the finding that DDT is adsorbing by solids in suspension in the water, greater effectiveness can be achieved when the river is relatively turbid.

It is believed that similar treatments would be effective for long distances in silty streams other than the Saskatchewan River, and the experience in Saskatchewan also suggests that improved control would be obtained in small streams with clear water by the addition of finely divided inorganic material with marked DDT adsorptive properties, or by treating with such material with the DDT already incorporated into it.

In the black-fly control tests on the North and South Saskatchewan rivers the oil solution of DDT was applied by aircraft (1), from 45-gallon barrels located on a bridge or ferry and from 5-gallon containers on a bridge. All methods were about equally effective in obtaining a uniform dispersion of larvicide across the river, but the barrel method was the simplest. With the barrel method the resultant oil film, originating in the centre of the

river, spread rapidly across its entire width. Calibration of application equipment was unnecessary. The rate of discharge from a faucet was measured and the calculated quantity of larvicide that would pour out in 15 minutes was placed in the barrel. It was necessary to calculate only the total quantity of larvicide required to provide 0.1 p.p.m. of DDT for 15 minutes. The method of application is therefore relatively simple and there appears to be no need for special applicator apparatus like that used by Hocking (7) for small streams.

ACKNOWLEDGMENTS

The authors wish to acknowledge the invaluable assistance of the following people, each of whom contributed most willingly of his special knowledge and abilities: C. R. Twinn, Head, Veterinary and Medical Entomology Unit, Ottawa; J. W. T. Spinks, Dean, Graduate School, and Head, Department of Chemistry, University of Saskatchewan; H. R. Sallans, Prairie Regional Laboratory, National Research Council, Saskatoon; and V. E. Kinsey, Harvard School of Medicine, Boston, Mass.

The assistance of members of the Veterinary and Medical Entomology Unit at Saskatoon, particularly of A. R. Murray in 1949, and of J. R. Anderson and W. M. Hallett in 1950, in field and laboratory work, is also gratefully acknowledged.

REFERENCES

1. Arnason, A. P., A. W. A. Brown, F. J. H. Fredeen, W. W. Hopewell, and J. G. Rempel. Experiments in the control of *Simulium arcticum* Malloch by means of DDT in the Saskatchewan River. Sci. Agr. 29 : 527-537. 1949.
2. Berck, B. Microdetermination of DDT in river water and suspended solids. Anal. Chem. *In preparation.*
3. Fairchild, G. B., and E. A. Barreda. DDT as a larvicide against *Simulium*. J. Econ. Ent. 38 : 694-699. 1945.
4. Garnham, P. C. C., and J. P. McMahon. The eradication of *Simulium neavei* Roubaud from an onchocerciasis area in Kenya Colony. Bull. Ent. Res. 37 : 619-628. 1947.
5. Gjullin, C. M., O. B. Cope, B. F. Quisenberry, and F. R. DuChanois. The effect of some insecticides on black-fly larvae in Alaskan streams. J. Econ. Ent. 42 : 100-105. 1949.
6. Hocking, B., C. R. Twinn, and W. C. McDuffie. A preliminary evaluation of some insecticides against immature stages of blackflies. Sci. Agr. 29 : 69-80. 1949.
7. Hocking, B. Further tests of insecticides against black flies (Diptera : Simuliidae) and a control procedure. Sci. Agr. 30 : 489-508. 1950.
8. McBryde, W. A. E. Photometric analysis error. Anal. Chem. 24 : 1639-1640. 1952.
9. Schechter, M. S., S. B. Soloway, R. A. Hayes, and H. L. Haller. Colorimetric determination of DDT. Ind. Eng. Chem., *Anal Ed.* 17 : 704-709. 1950.
10. Twinn, C. R. Studies of the biology and control of biting flies in northern Canada. Arctic 3 : 14-26. 1950.
11. Twinn, C. R. A review of studies of blood-sucking flies in northern Canada. Canadian Ent. 84 : 22-28. 1952.

A FOUR-ROW PLOT SEEDER[1]

W. E. Smith, J. E. Fitzsimmons and J. Rawlings[2]

University of Alberta, Edmonton, Alta.

[Received for publication October 27, 1952]

ABSTRACT

Improvements and modifications of the Grafius type four-row small grain nursery seeder are described. The machine is similar in construction to the Grafius type seeder but differs mainly in that it has a centrifugal type of divider similar in operation to the cyclone grass seeder. The seeding unit is mounted on a 5-horsepower riding-type garden tractor which can also be used for cultivation.

INTRODUCTION

A number of four-row plot seeders have been developed (1, 2, 3) in attempts to reduce the time and labour involved in seeding experimental field plots. Some years ago the present authors constructed a seeder capable of continuous seeding, similar to that described by Grafius (1). Although this type of seeder permitted continuous operation, several disadvantages were encountered, as follows: *first*, the gravity divider was not dependable under all field conditions; *second*, the flexible cable used to drive the Kemp "V" belt was not sufficiently positive in its drive to prevent variations in belt speed; and *third*, the garden tractor used had insufficient traction and was difficult to control.

With these objections in mind a new machine was constructed, using centrifugal force instead of gravity to distribute the seed evenly in the four rows. A riding-type garden tractor was used and the Kemp "V" belt was driven by a belt from a pulley attached to a ground wheel of the tractor.

DESCRIPTION OF THE MACHINE

The assembled machine is shown in Figure 1. The 5-horsepower tractor is a standard machine. The double disk furrow openers with arms and springs are the standard types used for most grain drills. The divider shown in its mounted position below the superstructure in Figure 1 is illustrated in more detail in Figures 2, 3 and 4. In the operation, seed is dropped from the Kemp "V" belt into the top of a Y-shaped one-inch tube as shown in Figure 2. This tube is mounted on the shaft of a six-volt car heater motor as shown in Figure 3. This tube and motor assembly is mounted inside the divider box just above the four divisions as shown in Figure 4. The seed is spread evenly in the divider box by the centrifugal force of the revolving tube. The box is divided into four equal separations that allow for an equal portion of seed to go continuously into each of the four rows.

The power used to drive the motor is derived from three $1\frac{1}{2}$-volt dry cell batteries mounted under the tractor seat support, as shown in Figure 1. The speed of the motor can be controlled by a variable speed switch similar to that found on many heaters.

[1] Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alta.
[2] Assistant Professor, Farm Superintendent, and Farm Mechanic, respectively.

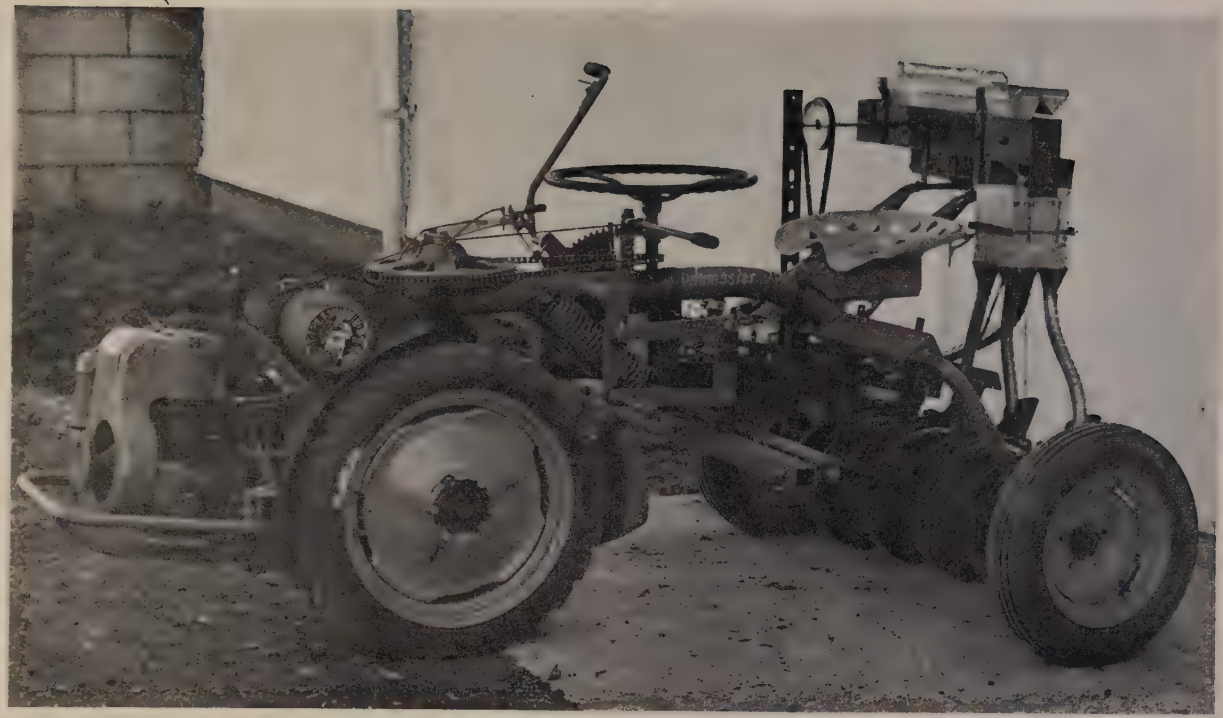


FIGURE 1. Side view of assembled machine.

FIGURE 2 (*Left*). Top view of centrifugal divider with cover removed.

FIGURE 3 (*Right*). Side view of rotating tube assembly.

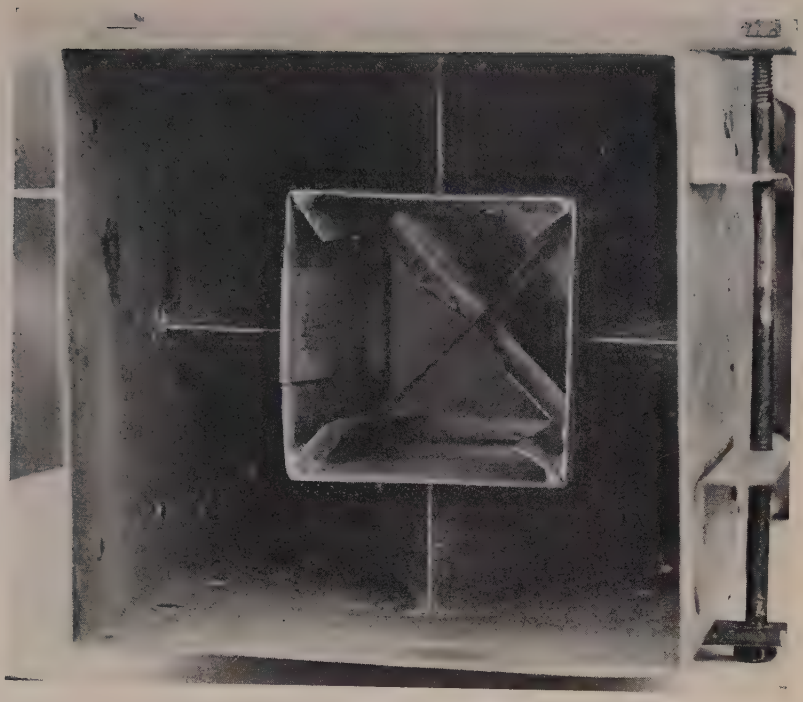


FIGURE 4. Top view of divider box with cover and rotating assembly removed.

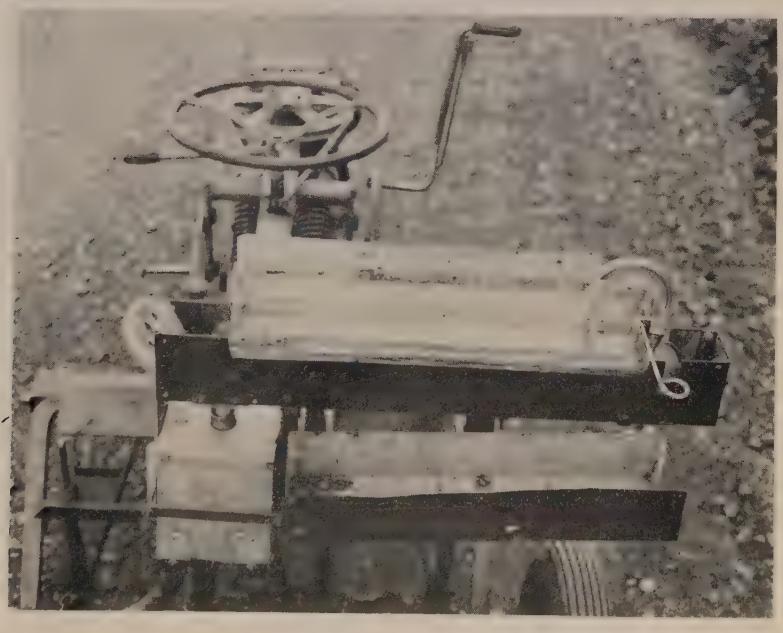


FIGURE 5. Top view of superstructure, showing position of the hinged hopper and assembled divider.

A hinged-hopper is mounted on the side of the superstructure as shown in Figure 5. This permits the operator to observe the Kemp "V" belt at all times.

The seeding procedure, as described by Grafius (1), has been closely followed in testing this machine and has proved to be satisfactory.

DISCUSSION

This machine has worked well on level, fairly well-packed soil; but trouble with traction has been encountered on very loose soil and grades greater than 5 per cent. The authors believe that if this unit were adapted to a tractor similar to that described by Magee (3) with packers, these difficulties would be eliminated. It is planned to put a large hopper with a suitable feeding device directly above the Kemp "V" belt, to permit the continuous seeding of large plots with the one variety.

REFERENCES

1. Grafius, J. E. A four-row nursery seeder. Agron. J. 41 : 267-269. 1949.
2. Kusch, A. G., *et al.* A four-row power plot seeder. Sci. Agr. 30 : 201-208. 1950.
3. Magee, A. I. Tractor-mounted four-row plot seeder. Sci. Agr. 31 : 120-121. 1951.

THE EFFECT OF MONTH AND SEASON UPON BREEDING EFFICIENCY OBTAINED WITH ARTIFICIAL INSEMINATION UNDER ONTARIO CONDITIONS

T. D. Burgess[1]

Department of Animal Husbandry, Ontario Agricultural College, Guelph, Ont.

[Received for publication October 23, 1952]

ABSTRACT

The effect of month and season upon the per cent non-return obtained with artificial insemination in Ontario has been studied using the monthly per cent non-return at 60 to 90 days obtained by six licensed Ontario units in the years 1948 to 1952. This includes some 328,295 first services in all. The seasons were taken as follows:

Spring — March to May, inclusive;
Summer — June to August, inclusive;
Fall — September to November, inclusive, and
Winter — December to February, inclusive.

No significant difference was found that could be attributed to either months or seasons.

INTRODUCTION

There is a belief among livestock men that it is easier to get cows in calf at certain times of the year than at others. This belief is important when viewed with respect to artificial insemination in the Province. If there are certain periods of the year when it is more difficult to settle cows than others, then it may necessitate a change in practice at the artificial insemination units during these periods.

There is a large volume of literature on this subject. The majority of the studies have been carried out in the United States. Mercier (7) reporting on three Canadian herds found the highest fertility in the winter. Erb reporting on twenty years' records found that the highest fertility occurred in May and the lowest in August. Hilder (4) reports the lowest fertility in mid-summer and this was followed by a sharp increase in the fall. Morgan (8) reporting on breeding records covering a 38-year period found that the highest fertility occurred in December and the lowest occurred in September. Asdell (1) reports the lowest fertility in January and the highest in June. Trimberger (10) found summer and late summer to have the lowest fertility. Perry (9) states that the lowest breeding efficiency occurs in July and August and the highest in May and June. Lewis (5) found the peak breeding efficiency for Holsteins to be in March and April while for Guernseys it occurred in October and November. A lower efficiency was found for both breeds in winter and summer. Bonadonna (2) found that in Italy the highest rate of conception was obtained from January to June.

In view of the inconsistencies found in the literature, it was thought advisable to investigate this problem in the light of Ontario conditions. For this reason a study was undertaken to determine the following:

1. The effect of the different seasons on the fertility level obtained in artificial insemination in Ontario.
2. The effect of different months on the fertility level obtained in artificial insemination in Ontario.

[1] The data are taken from a thesis submitted by the author in partial fulfilment of the degree of Master of Science in Agriculture 1952.

MATERIALS AND METHODS

The data for this study were drawn from the "Breeding Efficiency Reports of the Ontario Livestock Branch" for the period from March 1948 to February 1952. These reports give the number of first services and non-returns to service at 60 to 90 days for each licensed unit in Ontario for each month. In the case of the investigation of monthly differences the reports from January 1949 to December 1951 only were used in order to give three complete years as a report for January and February of 1948 were not available. The study for monthly variation uses 263,584 first services and that for seasons 328,295. In this study the seasons were taken as follows:

Spring — March to May, inclusive;
Summer — June to August, inclusive;
Fall — September to November, inclusive, and
Winter — December to February, inclusive.

The per cent non-returns at 60 to 90 days were used as the measure of fertility. These percentages were calculated for each month and each season. These percentages were then analysed by Fisher's analysis of various methods.

EXPERIMENTAL RESULTS

The per cent non-returns at 60 to 90 days for each season are presented in Table 1.

TABLE 1.—PER CENT NON-RETURNS FOR LICENSED ONTARIO UNITS FOR EACH SEASON FOR THE YEARS 1948-49 TO 1951-52

Season	Per cent non-returns at 60-90 days			
	1948-49	1949-50	1950-51	1951-52
Spring	64.3	61.7	68.8	69.6
Summer	61.1	59.1	70.9	71.0
Fall	66.3	67.5	70.2	70.2
Winter	64.8	66.6	68.9	67.0

The per cent non-returns at 60 to 90 days for each month for the years 1949, 1950, 1951 are presented in Table 2.

When these data were analysed no significant difference was found for either seasons or months. There is, however, a significant difference between years. The years 1950 and 1951 have a significantly higher rate of non-returns than does 1949. Although 1951 has a higher non-return rate than 1950 the difference is not statistically significant.

DISCUSSION AND CONCLUSIONS

The effect of months and seasons upon the non-return rate at 60 to 90 days in Ontario Artificial Insemination Units has been studied. No significant difference was observed due to either seasons or months. These

TABLE 2.—PER CENT NON-RETURNS AT 60 TO 90 DAYS FOR THE LICENSED ONTARIO UNITS FOR EACH MONTH FOR THE YEARS 1949, 1950, 1951

Month	Per cent non-returns		
	1949	1950	1951
January	64.5	68.8	69.6
February	63.6	68.7	70.4
March	63.8	71.0	71.0
April	62.4	69.3	72.1
May	60.0	67.0	69.1
June	56.9	70.0	71.2
July	59.9	71.9	71.2
August	64.7	72.0	72.3
September	63.8	69.9	71.2
October	69.5	72.2	71.8
November	68.8	68.9	68.1
December	63.2	67.0	67.4

results are at variance with other writers. However, the other studies reported in the literature were conducted in places other than Ontario where management practices and climate would vary to some extent.

A significant difference was found between years, 1950 and 1951 having a significantly higher non-return rate than 1949. This may be due to increasing efficiency as the units gain experience or it might be due to some difference between the years. The specific cause for the yearly variation could not be ascertained from the data at hand.

REFERENCES

1. Asdell, S. A. Sterility and delayed breeding in dairy cattle. Cornell Ext. Bull. 737. 1948.
2. Bonadonna, T. Artificial insemination and fertility in relation to time of year. An. Breeding Abs. Vol. 19, No. 1.
3. Erb, R. E. Some factors influencing breeding efficiency in dairy cattle. J. Dairy Sci. 25 : 815. 1942.
4. Hilder, R. H. The relationship of various factors to the breeding efficiency of dairy cattle and to the sex ratio of the offspring. J. Dairy Sci. 27 : 981-992. 1944.
5. Lewis, R. C. Effect of season upon spermotogenisis and fertility of dairy bulls under Michigan conditions. J. Animal Sci. 7 : 514.
6. Mercier, E. Seasonal effect in spermatzoan activity in the bull and reproduction in cattle. J. Dairy Sci. 29 : 556. 1946.
7. Mercier, E. Seasonal variations in hours of daylight associated with fertility level of cattle under natural breeding conditions. J. Dairy Sci. 30 : 747. 1947.
8. Morgan, R. F. Influence of age of dairy cattle and season of the year on the sex ratio of calves and services required for conception. Neb. Agric. Research Bull. 104. 1938.
9. Perry, E. J. The artificial insemination of farm animals. Rutgers University Press.
10. Trimberger, G. W. Predictability of breeding efficiency in dairy cattle from their previous conception rate and their heredity. J. Dairy Sci. 28 : 659. 1945.

A NEW TECHNIQUE FOR POLLEN GRAIN STUDY IN THE GRAMINEAE

J. W. Morrison[1]

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

[Received for publication November 10, 1952]

ABSTRACT

For a study of the gametophytic divisions in the pollen grains of *Triticum* and related grasses the following procedure has proved satisfactory: (A) Anthers at the correct stage are selected by squashing in aceto-carmine. (B) Pre-treatment for 2 hours in monobromonaphthalene to contract chromosomes and disorganize the spindle. (C) Fixation in Carnoy's fluid A overnight. (D) Hydrolyzing for 13 minutes, then staining in leuco-basic fuchsin for 2–3 hours followed by an intensifying period in tap water.

In cereals and grasses both gametophytic divisions take place within the pollen grain before anthesis. The first division produces a vegetative and a generative nucleus. The generative nucleus later divides to produce the two sperm nuclei which become the two male gametes. The pollen grains develop in a similar environment under maternal influence but, as a consequence of crossing over and recombination in meiosis, each pollen grain has its own distinctive genotype. However, few studies have been made of cereal pollen mitoses because of two major cytological difficulties: (1) The pollen grains are not synchronized in division at either the first or second mitosis; (2) The metabolic products of the cell (starch and proteins) or the cellulose wall absorb the stain and prevent clear definition of the contents. The method outlined below overcomes to a considerable degree the latter problem and somewhat alleviates the former.

The two most recognized methods used to examine pollen grains in the Gramineae are fixation in acetic-alcohol fixatives and staining in aceto-carmine, or embedding the anthers in paraffin and staining with the usual reagents [for the formulae of all reagents see Darlington and La Cour, (1)]. Fairly good results occasionally can be obtained with aceto-carmine, especially if care is taken in heating and pressing and if the number of chromosomes is small. Usually, however, the cell wall and cytoplasm stain nearly as darkly as the chromosomes. When the generative nucleus is dividing, the pollen grain is filled with starch. Aceto-carmine will stain the resting nucleus and sperms but it is very difficult, if not impossible, to study the mitotic division itself. Other acetic stains, orcein and lacmoid, are less useful than aceto-carmine. Sectioning does not prove satisfactory when details such as chromosome fragments are involved.

Most of the remarks and observations recorded here apply in particular to the wheat species and related genera, *Agropyron* and *Aegilops*. This method also gave satisfactory results in *Secale cereale*, *Zea mays* and a *Festuca-Lolium* hybrid. A slight modification may be necessary for other material.

[1] Now with Cereal Division, Central Experimental Farm, Ottawa, Canada.

PROCEDURE

A. Selection of anthers.
B. Pre-treatment if required.
C. Fixation.
D. Staining and mounting.

A. Selection of anthers.

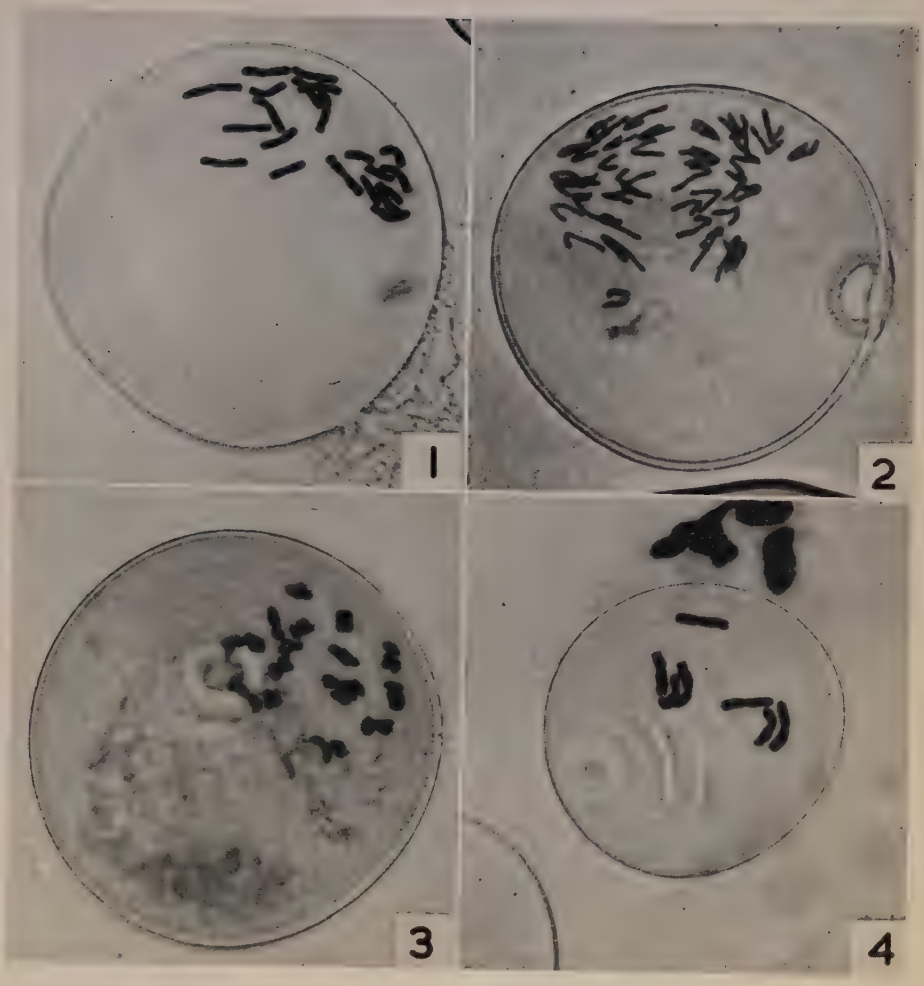
In wheat and its hybrids, the first gametophytic division occurs at a time when the spike is just emerging from the sheath. In some *Agropyron* species the head is well emerged before divisions occur. There is a gradation in maturity from primary to secondary and to tertiary florets of a spikelet and from the central spikelet to either end of the spike. At the time of division there is no visible starch in the pollen grain and the cell is highly vacuolated. An examination of one anther from a floret will determine the age of the anthers and whether divisions are taking place. There is usually no more than 5 per cent of the pollen grains undergoing division at any one time because the pollen grains within one anther are not synchronized in division. Between the three anthers there is also some variation in age and thus in the number of mitoses at metaphase. Divisions occur at all hours of the day in no special cycle. Mid-day fixation gave satisfactory results.

The division of the generative nucleus (Figure 3) occurs approximately four days after the first division. At this time the head is usually completely emerged from the sheath. The pollen grains at this time are filled with starch granules and often only one nucleus is visible in the temporary squash because the vegetative nucleus is very weakly stained. It may be necessary to heat the slide repeatedly before mitosis can be seen in the preliminary aceto-carmine squash. A high magnification is usually required to be certain that divisions are occurring.

B. Pre-treatment

For studies of some of the mitotic stages pre-treatment is not advisable (Figure 2). For counting and for an examination of the morphology of the chromosomes and fragments, it is necessary to disorganize the spindle and shorten the chromosomes (Figures 1, 3, and 4). The author has used (*a*) a saturated solution of monobromonaphthalene for two to four hours (3); (*b*) 0.05 per cent colchicine for three to four hours; and to a limited extent (*c*) 0.002 *M* 8-oxyquinoline for three hours (4). Besides being cheaper, monobromonaphthalene gave as good results as colchicine. The optimum length of pre-treatment varies with the temperature, which must be high enough to keep the pollen grains viable. When the anthers are dropped into a vial of saturated monobromonaphthalene they do not sink immediately. They can be made to do so by pressing them down with a needle or expelling the air from them by squeezing them on the sides of the glass. The use of a wetting agent did not improve the technique.

Pre-treatment is an essential feature of the method. With chromosomes contracted and scattered it is possible to count clearly up to 50 chromosomes in a pollen grain or to distinguish fragmented chromosomes, telocentrics, etc. At the same time there is a slight accumulation of metaphases.



Photomicrographs of *Triticum* pollen grains, 1120×.

FIGURE 1. Metaphase, *T. vulgare*, pre-treated with monobromonaphthalene.

FIGURE 2. Anaphase, *T. vulgare*, no pre-treatment.

FIGURE 3. Metaphase, division of the generative nucleus, 17 chromosomes in F_1 pentaploid (*T. vulgare* × *T. durum*).

FIGURE 4. Metaphase, *T. monococcum*, pre-treated.

C. *Fixation*

After the required time of pre-treatment the anthers are fixed overnight in Carnoy's fluid 6 : 3 : 1. They can be left for several days in the fixative without harm. Acetic-alcohol fixation can also be used. Osmic fixatives tend to colour the cell wall.

D. *Staining and mounting*

After pre-treatment and storage in Carnoy's reagent, staining in aceto-carmine gives fair results but not in any way comparable to Feulgen. The acid hydrolysis clears the cytoplasm and leaves resting nuclei or chromosomes plainly visible. The time of hydrolysis varies from nine to fifteen minutes, with an optimum at thirteen, which is an increase over the normal time [cf. Hillary, (2)]. After hydrolysis the anthers are stained in leuco-basic-fuchsin for two to three hours. Frequently the staining will be light but it can be greatly intensified by soaking the anthers in tap water for an hour or longer. After staining the anthers are softened in 45 per cent acetic acid and then squashed. The debris is removed from the slide and a cover slip applied. When the pollen grains are reaching maturity slight pressure will rupture them, but at the time of the first division more pressure can be exerted on the cover slip.

Slides can be made permanent by removing the cover slip in 45 per cent acetic acid and passing them through the alcohol series, 80 per cent and two changes of absolute, and then mounting in euparal. There are two difficulties encountered which limit the usefulness of this procedure and suggest the use of temporary mounts; (*a*) the loss of the pollen grains from the cover slip and slide which can be partly overcome by the use of albumen and by careful transference through the alcohol series; (*b*) as soon as pressure is released the pollen grains are free to change shape and the walls will wrinkle. Unless the slides are again pressed before the euparal has hardened this wrinkling will obscure some details of the chromosomes. Temporary mounts can be kept for a week or two by ringing the cover slip with vaseline or a rubber cement solution.

ACKNOWLEDGMENT

The advice and criticism of L. F. La Cour, and the financial assistance of the National Research Council of Canada, are gratefully acknowledged.

REFERENCES

1. Darlington, C. D., and L. F. La Cour. The handling of chromosomes. Macmillan & Co., New York. 1947.
2. Hillary, B. B. Use of the Feulgen reaction in cytology. Bot. Gaz. 101 : 276–300. 1939.
3. O'Mara, J. G. Acetic acid methods for chromosome studies at prophase and metaphase in meristems. Stain Tech. 23 : 201-204. 1948.
4. Tjio, J. H., and A. Levan. The use of oxyquinoline in chromosome analysis. An. Estac. Exp. Aula Dei 2 : 21–64. 1950.

SELECTIVE CONTROL OF WILD OATS IN CEREAL CROPS BY MALEIC HYDRAZIDE

GEORGE KNOWLES

Division of Field Husbandry, Soils and Agricultural Engineering, Central Experimental Farm, Ottawa, Canada

[Received for publication March 7, 1953]

The margin of difference in the time at which some cereal crops reach the heading stage of growth, and the time at which wild oats (*A. fatua*) reaches this stage, may make possible the selective control of this weed in these crops by the application of maleic hydrazide.

In 1952, at the Central Experimental Farm, Ottawa, wild oats and cereal crops were rendered almost completely sterile by spraying before heading with a solution of maleic hydrazide plus a wetting agent at the rate of one pound of active ingredient per acre. The heads emerged but they contained hardly any seed. However, a later treatment of exactly the same amount of maleic hydrazide, applied when the barley and wheat had been in head for six days but while the wild and cultivated oats were still only in the "shot blade" stage, caused almost complete sterility of the wild and cultivated oats but did not materially affect the yields of barley and wheat.

Nine per cent of the barley heads, 16 per cent of the wheat heads, 40 per cent of the flax and almost 100 per cent of the cultivated and wild oat heads were sterile. A germination test was made of the seeds collected from the plots which received this later treatment. The germination of the barley and flax seeds was not affected by the treatment. Wheat seed from the treated plots germinated only 9 per cent compared with 92 per cent germination of the wheat seeds from the untreated plots. A very few wild oat seeds were obtained from the treated plots and of these the germination was 6.9 per cent, compared with 62.4 per cent germination for the wild oats seed from the untreated area.

Maleic hydrazide is a new chemical and has not yet been registered for use in Canada. More complete tests of its value in controlling wild oats will be carried out on Experimental Farms located in areas where this weed is a serious problem. The low germination of wheat seed from plots treated with maleic hydrazide after the wheat had headed may be a serious drawback to the use of this chemical.

FIGURE 1. On the *left*, untreated check. On the *right*, heads of wheat made sterile by applying maleic hydrazide before pollination.

NOTE ON EXPERIMENTS ON THE NUTRITIONAL VALUE OF "FEED-ANI"

A commercial product bearing the trade name "Feed-Ani" has been widely sold in Western Canada in recent months. This feed supplement appears to be rather costly but has been claimed to effect striking improvements in a wide variety of livestock and poultry problems and ailments. Since these claims have been based largely on testimonial letters rather than well-controlled experiments, investigations of some aspects of the product were made at Saskatoon. The studies were made with chicks and with mice. The experiments with mice covered a wide range of diets in which vitamin adequacy, protein quality and basal ingredients varied considerably. It is believed that the conclusions drawn would apply generally to chick growth and to growth in non-ruminants, such as pigs.

We wish to submit the concluding paragraph of an article describing these experiments on "Feed-Ani". The complete report will be published in an early issue of this Journal.

"From the standpoint of economics, the increased feed cost due to inclusion of 2 per cent 'Feed-Ani', together with the decreased feed efficiency, resulted in an over-all increase in cost per unit gain of 15 to 20 per cent over the cost when using an ordinary commercial ration. There is thus little indication that 'Feed-Ani' is of value in the rations of growing chicks or non-ruminant farm animals when it is included at recommended levels in otherwise adequate rations based largely on Western Canadian grains."

—J. B. O'Neil and J. M. Bell,

June 1, 1953 University of Saskatchewan, Saskatoon, Sask.